

## Albumin Fusion Proteins

This application claims the benefit of priority under 35 U.S.C. § 119(e) based on the following U.S. provisional applications: 60/229,358 filed on April 12, 2000; 60/199,384 filed on April 25, 2000; and 60/256,931 filed on December 21, 2000. Each of the provisional applications is hereby incorporated by reference in its entirety.

### BACKGROUND OF THE INVENTION

The invention relates generally to Therapeutic proteins (including, but not limited to, a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or fragments or variants of albumin. The invention further relates to Therapeutic proteins (including, but not limited to, a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or fragments or variants of albumin, that exhibit extended shelf-life and/or extended or therapeutic activity in solution. These fusion proteins are herein collectively referred to as "albumin fusion proteins of the invention." The invention encompasses therapeutic albumin fusion proteins, compositions, pharmaceutical compositions, formulations and kits. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention using these nucleic acids, vectors, and/or host cells.

The invention is also directed to methods of *in vitro* stabilizing a Therapeutic protein via fusion or conjugation of the Therapeutic protein to albumin or fragments or variants of albumin.

Human serum albumin (HSA, or HA), a protein of 585 amino acids in its mature form (as shown in Figure 15 or in SEQ ID NO:18), is responsible for a significant proportion of the osmotic pressure of serum and also functions as a carrier of endogenous and exogenous ligands. At present, HA for clinical use is produced by extraction from human blood. The production of recombinant HA (rHA) in microorganisms has been disclosed in EP 330 451 and EP 361 991.

The role of albumin as a carrier molecule and its inert nature are desirable

properties for use as a carrier and transporter of polypeptides *in vivo*. The use of albumin as a component of an albumin fusion protein as a carrier for various proteins has been suggested in WO 93/15199, WO 93/15200, and EP 413 622. The use of N-terminal fragments of HA for fusions to polypeptides has also been proposed (EP 399 666). Fusion of albumin to the Therapeutic protein may be achieved by genetic manipulation, such that the DNA coding for HA, or a fragment thereof, is joined to the DNA coding for the Therapeutic protein. A suitable host is then transformed or transfected with the fused nucleotide sequences, so arranged on a suitable plasmid as to express a fusion polypeptide. The expression may be effected *in vitro* from, for example, prokaryotic or eukaryotic cells, or *in vivo* e.g. from a transgenic organism.

Therapeutic proteins in their native state or when recombinantly produced, such as interferons and growth hormones, are typically labile molecules exhibiting short shelf-lives, particularly when formulated in aqueous solutions. The instability in these molecules when formulated for administration dictates that many of the molecules must be lyophilized and refrigerated at all times during storage, thereby rendering the molecules difficult to transport and/or store. Storage problems are particularly acute when pharmaceutical formulations must be stored and dispensed outside of the hospital environment. Many protein and peptide drugs also require the addition of high concentrations of other protein such as albumin to reduce or prevent loss of protein due to binding to the container. This is a major concern with respect to proteins such as IFN. For this reason, many Therapeutic proteins are formulated in combination with large proportion of albumin carrier molecule (100-1000 fold excess), though this is an undesirable and expensive feature of the formulation.

Few practical solutions to the storage problems of labile protein molecules have been proposed. Accordingly, there is a need for stabilized, long lasting formulations of proteinaceous therapeutic molecules that are easily dispensed, preferably with a simple formulation requiring minimal post-storage manipulation.

#### SUMMARY OF THE INVENTION

The present invention is based, in part, on the discovery that Therapeutic proteins may be stabilized to extend the shelf-life, and/or to retain the Therapeutic protein's activity for extended periods of time in solution, *in vitro* and/or *in vivo*, by genetically or

chemically fusing or conjugating the Therapeutic protein to albumin or a fragment (portion) or variant of albumin, that is sufficient to stabilize the protein and/or its activity. In addition it has been determined that the use of albumin-fusion proteins or albumin conjugated proteins may reduce the need to formulate protein solutions with large excesses of carrier proteins (such as albumin, unfused) to prevent loss of Therapeutic proteins due to factors such as binding to the container.

The present invention encompasses albumin fusion proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or a fragment (portion) or variant of albumin. The present invention also encompasses albumin fusion proteins comprising a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) fused to albumin or a fragment (portion) or variant of albumin, that is sufficient to prolong the shelf life of the Therapeutic protein, and/or stabilize the Therapeutic protein and/or its activity in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo*. Nucleic acid molecules encoding the albumin fusion proteins of the invention are also encompassed by the invention, as are vectors containing these nucleic acids, host cells transformed with these nucleic acids vectors, and methods of making the albumin fusion proteins of the invention and using these nucleic acids, vectors, and/or host cells.

The invention also encompasses pharmaceutical formulations comprising an albumin fusion protein of the invention and a pharmaceutically acceptable diluent or carrier. Such formulations may be in a kit or container. Such kit or container may be packaged with instructions pertaining to the extended shelf life of the Therapeutic protein. Such formulations may be used in methods of treating, preventing, ameliorating or diagnosing a disease or disease symptom in a patient, preferably a mammal, most preferably a human, comprising the step of administering the pharmaceutical formulation to the patient.

In other embodiments, the present invention encompasses methods of preventing treating, or ameliorating a disease or disorder. In preferred embodiments, the present invention encompasses a method of treating a disease or disorder listed in the "Preferred Indication Y" column of Table 1 comprising administering to a patient in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Therapeutic protein portion corresponding to a Therapeutic protein (or

fragment or variant thereof) disclosed in the "Therapeutic Protein X" column of Table 1 (in the same row as the disease or disorder to be treated is listed in the "Preferred Indication Y" column of Table 1) in an amount effective to treat prevent or ameliorate the disease or disorder.

5 In another embodiment, the invention includes a method of extending the shelf life of a Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) comprising the step of fusing or conjugating the Therapeutic protein to albumin or a fragment (portion) or variant of albumin, that is sufficient to extend the shelf-life of the Therapeutic protein. In a preferred embodiment, the Therapeutic protein used according to this method is fused to the albumin, or the fragment or variant of albumin. In 10 a most preferred embodiment, the Therapeutic protein used according to this method is fused to albumin, or a fragment or variant of albumin, via recombinant DNA technology or genetic engineering.

In another embodiment, the invention includes a method of stabilizing a 15 Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) in solution, comprising the step of fusing or conjugating the Therapeutic protein to albumin or a fragment (portion) or variant of albumin, that is sufficient to stabilize the Therapeutic protein. In a preferred embodiment, the Therapeutic protein used according to this method is fused to the albumin, or the fragment or variant of albumin. In a most 20 preferred embodiment, the Therapeutic protein used according to this method is fused to albumin, or a fragment or variant of albumin, via recombinant DNA technology or genetic engineering.

The present invention further includes transgenic organisms modified to contain the nucleic acid molecules of the invention, preferably modified to express the albumin 25 fusion proteins encoded by the nucleic acid molecules.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 depicts the extended shelf-life of an HA fusion protein in terms of the biological activity (Nb2 cell proliferation) of HA-hGH remaining after incubation in cell 30 culture media for up to 5 weeks at 37°C. Under these conditions, hGH has no observed activity by week 2.

Figure 2 depicts the extended shelf-life of an HA fusion protein in terms of the



stable biological activity (Nb2 cell proliferation) of HA-hGH remaining after incubation in cell culture media for up to 3 weeks at 4, 37, or 50°C. Data is normalized to the biological activity of hGH at time zero.

Figures 3A and 3B compare the biological activity of HA-hGH with hGH in the Nb2 cell proliferation assay. Figure 3A shows proliferation after 24 hours of incubation with various concentrations of hGH or the albumin fusion protein, and Figure 3B shows proliferation after 48 hours of incubation with various concentrations of hGH or the albumin fusion protein.

Figure 4 shows a map of a plasmid (pPPC0005) that can be used as the base vector into which polynucleotides encoding the Therapeutic proteins (including polypeptide and fragments and variants thereof) may be cloned to form HA-fusions. Plasmid Map key: PRB1p: *PRB1 S. cerevisiae* promoter; FL: Fusion leader sequence; rHA: cDNA encoding HA; ADH1t: *ADH1 S. cerevisiae* terminator; T3: T3 sequencing primer site; T7: T7 sequencing primer site; Amp R:  $\beta$ -lactamase gene; ori: origin of replication. Please note that in the provisional applications to which this application claims priority, the plasmid in Figure 4 was labeled pPPC0006, instead of pPPC0005. In addition the drawing of this plasmid did not show certain pertinent restriction sites in this vector. Thus in the present application, the drawing is labeled pPPC0005 and more restriction sites of the same vector are shown.

Figure 5 compares the recovery of vial-stored HA-IFN solutions of various concentrations with a stock solution after 48 or 72 hours of storage.

Figure 6 compares the activity of an HA- $\alpha$ -IFN fusion protein after administration to monkeys via IV or SC.

Figure 7 describes the bioavailability and stability of an HA- $\alpha$ -IFN fusion protein.

Figure 8 is a map of an expression vector for the production of HA-IFN.

Figure 9 shows the location of loops in HA.

Figure 10 is an example of the modification of an HA loop.

Figure 11 is a representation of the HA loops.

Figure 12 shows the HA loop IV.

Figure 13 shows the tertiary structure of HA.

Figure 14 shows an example of a scFv-HA fusion

Figure 15 shows the amino acid sequence of the mature form of human albumin

(SEQ ID NO:18) and a polynucleotide encoding it (SEQ ID NO:17).

#### DETAILED DESCRIPTION

As described above, the present invention is based, in part, on the discovery that a  
5 Therapeutic protein (e.g., a polypeptide, antibody, or peptide, or fragments and variants thereof) may be stabilized to extend the shelf-life and/or retain the Therapeutic protein's activity for extended periods of time in solution (or in a pharmaceutical composition) *in vitro* and/or *in vivo*, by genetically fusing or chemically conjugating the Therapeutic protein, polypeptide or peptide to all or a portion of albumin sufficient to stabilize the  
10 protein and its activity.

The present invention relates generally to albumin fusion proteins and methods of treating, preventing, or ameliorating diseases or disorders. As used herein, "albumin fusion protein" refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Therapeutic protein (or  
15 fragment or variant thereof). An albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Therapeutic protein is joined in-frame with a  
20 polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The Therapeutic protein and albumin protein, once part of the albumin fusion protein, may be referred to as a "portion", "region" or "moiety" of the albumin fusion protein (e.g., a "Therapeutic protein portion" or an "albumin protein portion").

In one embodiment, the invention provides an albumin fusion protein comprising,  
25 or alternatively consisting of, a Therapeutic protein (e.g., as described in Table 1) and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Therapeutic protein and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or  
30 alternatively consisting of, a biologically active and/or therapeutically active variant of a Therapeutic protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum

albumin.

In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein, and a biologically active and/or therapeutically active fragment of serum albumin. In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Therapeutic protein portion of the albumin fusion protein is the mature portion of the Therapeutic protein. In a further preferred embodiment, the Therapeutic protein portion of the albumin fusion protein is the extracellular soluble domain of the Therapeutic protein. In an alternative embodiment, the Therapeutic protein portion of the albumin fusion protein is the active form of the Therapeutic protein.

In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment or variant of a Therapeutic protein and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of a Therapeutic protein and the mature portion of serum albumin.

#### **Therapeutic proteins**

As stated above, an albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion or chemical conjugation.

As used herein, "Therapeutic protein" refers to proteins, polypeptides, antibodies, peptides or fragments or variants thereof, having one or more therapeutic and/or biological activities. Therapeutic proteins encompassed by the invention include but are not limited to, proteins, polypeptides, peptides, antibodies, and biologics. (The terms peptides, proteins, and polypeptides are used interchangeably herein.) It is specifically contemplated that the term "Therapeutic protein" encompasses antibodies and fragments and variants thereof. Thus an albumin fusion protein of the invention may contain at least a fragment or variant of a Therapeutic protein, and/or at least a fragment or variant of an antibody.

Additionally, the term "Therapeutic protein" may refer to the endogenous or naturally occurring correlate of a Therapeutic protein.

By a polypeptide displaying a "therapeutic activity" or a protein that is "therapeutically active" is meant a polypeptide that possesses one or more known biological and/or therapeutic activities associated with a therapeutic protein such as one or more of the Therapeutic proteins described herein or otherwise known in the art. As a non-limiting example, a "Therapeutic protein" is a protein that is useful to treat, prevent or ameliorate a disease, condition or disorder. As a non-limiting example, a "Therapeutic protein" may be one that binds specifically to a particular cell type (normal (e.g., lymphocytes) or abnormal e.g., (cancer cells)) and therefore may be used to target a compound (drug, or cytotoxic agent) to that cell type specifically.

In another non-limiting example, a "Therapeutic protein" is a protein that has a biological activity, and in particular, a biological activity that is useful for treating preventing or ameliorating a disease. A non-inclusive list of biological activities that may be possessed by a Therapeutic protein includes, enhancing the immune response, promoting angiogenesis, inhibiting angiogenesis, regulating hematopoietic functions, stimulating nerve growth, enhancing an immune response, inhibiting an immune response, or any one or more of the biological activities described in the "Biological Activities" section below.

As used herein, "therapeutic activity" or "activity" may refer to an activity whose effect is consistent with a desirable therapeutic outcome in humans, or to desired effects in non-human mammals or in other species or organisms. Therapeutic activity may be measured *in vivo* or *in vitro*. For example, a desirable effect may be assayed in cell culture. As an example, when hGH is the Therapeutic protein, the effects of hGH on cell proliferation as described in Example 1 may be used as the endpoint for which therapeutic activity is measured. Such *in vitro* or cell culture assays are commonly available for many Therapeutic proteins as described in the art. . Examples of assays include, but are not limited to those described herein in the Examples section or in the "Exemplary Activity Assay" column of Table 1.

Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, such as cell surface and secretory proteins, are often modified by the attachment of one or more oligosaccharide groups. The modification,

referred to as glycosylation, can dramatically affect the physical properties of proteins and can be important in protein stability, secretion, and localization. Glycosylation occurs at specific locations along the polypeptide backbone. There are usually two major types of glycosylation: glycosylation characterized by O-linked oligosaccharides, which are attached to serine or threonine residues; and glycosylation characterized by N-linked oligosaccharides, which are attached to asparagine residues in an Asn-X-Ser/Thr sequence, where X can be any amino acid except proline. N-acetylneuramic acid (also known as sialic acid) is usually the terminal residue of both N-linked and O-linked oligosaccharides. Variables such as protein structure and cell type influence the number and nature of the carbohydrate units within the chains at different glycosylation sites. Glycosylation isomers are also common at the same site within a given cell type.

For example, several types of human interferon are glycosylated. Natural human interferon- $\alpha$ 2 is O-glycosylated at threonine 106, and N-glycosylation occurs at asparagine 72 in interferon- $\alpha$ 14 (Adolf *et al.*, J. Biochem 276:511 (1991); Nyman TA *et al.*, J. Biochem 329:295 (1998)). The oligosaccharides at asparagine 80 in natural interferon- $\beta$ 1 $\alpha$  may play an important factor in the solubility and stability of the protein, but may not be essential for its biological activity. This permits the production of an unglycosylated analog (interferon- $\beta$ 1b) engineered with sequence modifications to enhance stability (Hosoi *et al.*, J. Interferon Res. 8:375 (1988; Karpusas *et al.*, Cell Mol Life Sci 54:1203 (1998); Knight, J. Interferon Res. 2:421 (1982); Runkel *et al.*, Pharm Res 15:641 (1998); Lin, Dev. Biol. Stand. 96:97 (1998))1. Interferon- $\gamma$  contains two N-linked oligosaccharide chains at positions 25 and 97, both important for the efficient formation of the bioactive recombinant protein, and having an influence on the pharmacokinetic properties of the protein (Sareneva *et al.*, Eur. J. Biochem 242:191 (1996); Sareneva *et al.*, Biochem J. 303:831 (1994); Sareneva *et al.*, J. Interferon Res. 13:267 (1993)). Mixed O-linked and N-linked glycosylation also occurs, for example in human erythropoietin, N-linked glycosylation occurs at asparagine residues located at positions 24, 38 and 83 while O-linked glycosylation occurs at a serine residue located at position 126 (Lai *et al.*, J. Biol. Chem. 261:3116 (1986); Broudy *et al.*, Arch. Biochem. Biophys. 265:329 (1988)).

Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, as well as analogs and variants thereof, may be modified

so that glycosylation at one or more sites is altered as a result of manipulation(s) of their nucleic acid sequence, by the host cell in which they are expressed, or due to other conditions of their expression. For example, glycosylation isomers may be produced by abolishing or introducing glycosylation sites, *e.g.*, by substitution or deletion of amino acid residues, such as substitution of glutamine for asparagine, or unglycosylated recombinant proteins may be produced by expressing the proteins in host cells that will not glycosylate them, *e.g.* in *E. coli* or glycosylation-deficient yeast. These approaches are described in more detail below and are known in the art.

Therapeutic proteins (particularly those disclosed in Table 1) and their nucleic acid sequences are well known in the art and available in public databases such as Chemical Abstracts Services Databases (*e.g.*, the CAS Registry), GenBank, and GenSeq as shown in Table 1.

Additional Therapeutic proteins corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention include, but are not limited to, one or more of the Therapeutic proteins or peptides disclosed in the "Therapeutic Protein X" column of Table 1, or fragment or variable thereof.

Table 1 provides a non-exhaustive list of Therapeutic proteins that correspond to a Therapeutic protein portion of an albumin fusion protein of the invention. The "Therapeutic Protein X" column discloses Therapeutic protein molecules followed by parentheses containing scientific and brand names that comprise, or alternatively consist of, that Therapeutic protein molecule or a fragment or variant thereof. "Therapeutic protein X" as used herein may refer either to an individual Therapeutic protein molecule (as defined by the amino acid sequence obtainable from the CAS and Genbank accession numbers), or to the entire group of Therapeutic proteins associated with a given Therapeutic protein molecule disclosed in this column. The "Exemplary Identifier" column provides Chemical Abstracts Services (CAS) Registry Numbers (published by the American Chemical Society) and/or Genbank Accession Numbers (*e.g.*, Locus ID, NP\_XXXXX (Reference Sequence Protein), and XP\_XXXXX (Model Protein) identifiers available through the national Center for Biotechnology Information (NCBI) webpage at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) that correspond to entries in the CAS Registry or Genbank database which contain an amino acid sequence of the Therapeutic Protein Molecule or of

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a fragment or variant of the Therapeutic Protein Molecule. In addition GenSeq Accession numbers and/or journal publication citations are given to identify the exemplary amino acid sequence for some polypeptides. The summary pages associated with each of these CAS and Genbank and GenSeq Accession Numbers as well as the cited journal publications (e.g., PubMed ID number (PMID)) are each incorporated by reference in their entireties, particularly with respect to the amino acid sequences described therein. The "PCT/Patent Reference" column provides U.S. Patent numbers, or PCT International Publication Numbers corresponding to patents and/or published patent applications that describe the Therapeutic protein molecule. Each of the patents and/or published patent applications cited in the "PCT/Patent Reference" column are herein incorporated by reference in their entireties. In particular, the amino acid sequences of the specified polypeptide set forth in the sequence listing of each cited "PCT/Patent Reference", the variants of these amino acid sequences (mutations, fragments, etc.) set forth, for example, in the detailed description of each cited "PCT/Patent Reference", the therapeutic indications set forth, for example, in the detailed description of each cited "PCT/Patent Reference", and the activity assays for the specified polypeptide set forth in the detailed description, and more particularly, the examples of each cited "PCT/Patent Reference" are incorporated herein by reference. The "Biological activity" column describes Biological activities associated with the Therapeutic protein molecule. The "Exemplary Activity Assay" column provides references that describe assays which may be used to test the therapeutic and/or biological activity of a Therapeutic protein or an albumin fusion protein of the invention comprising a Therapeutic protein X portion. Each of the references cited in the "Exemplary Activity Assay" column are herein incorporated by reference in their entireties, particularly with respect to the description of the respective activity assay described in the reference (see Methods section, for example) for assaying the corresponding biological activity set forth in the "Biological Activity" column of Table 1. The "Preferred Indication Y" column describes disease, disorders, and/or conditions that may be treated, prevented, diagnosed, or ameliorated by Therapeutic protein X or an albumin fusion protein of the invention comprising a Therapeutic protein X portion.

30 The recitation of "Cancer" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent,

and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., leukemias, cancers, and/or as described below under "Hyperproliferative Disorders").

In specific embodiments, a Therapeutic protein having a "Cancer" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a neoplasm located in a tissue selected from the group consisting of: colon, abdomen, bone, breast, digestive system, liver, pancreas, prostate, peritoneum, lung, blood (e.g., leukemia), endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), uterus, eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

In specific embodiments, a Therapeutic protein having a "Cancer" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a pre-neoplastic condition, selected from the group consisting of: hyperplasia (e.g., endometrial hyperplasia and/or as described in the section entitled "Hyperproliferative Disorders"), metaplasia (e.g., connective tissue metaplasia, atypical metaplasia, and/or as described in the section entitled "Hyperproliferative Disorders"), and/or dysplasia (e.g., cervical dysplasia, and bronchopulmonary dysplasia).

In another specific embodiment, a Therapeutic protein having a "Cancer" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a benign dysproliferative disorder selected from the group consisting of: benign tumors, fibrocystic conditions, tissue hypertrophy, and/or as described in the section entitled "Hyperproliferative Disorders".

The recitation of "Immune/Hematopoietic" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), blood disorders



(e.g., as described below under "Immune Activity" "Cardiovascular Disorders" and/or "Blood-Related Disorders"), and infections (e.g., as described below under "Infectious Disease").

5 In specific embodiments, a Therapeutic protein having a "Immune/Hematopoietic" recitation in the "Preferred Indication" column of Table 1, a fusion protein containing this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: anemia, pancytopenia, leukopenia, thrombocytopenia, leukemias, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL), plasmacytomas,  
10 multiple myeloma, Burkitt's lymphoma, arthritis, asthma, AIDS, autoimmune disease, rheumatoid arthritis, granulomatous disease, immune deficiency, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, immune reactions to transplanted organs and tissues, systemic lupus erythematosus, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, and allergies.

15 The recitation of "Reproductive" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), and disorders of the reproductive  
20 system (e.g., as described below under "Reproductive System Disorders").

In specific embodiments, a Therapeutic protein having a "Reproductive" recitation in the "Preferred Indication" column of Table 1, a fusion protein containing this Therapeutic protein, and fragments and variants thereof, may be used for example, to  
25 diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: cryptorchism, prostatitis, inguinal hernia, varicocele, leydig cell tumors, verrucous carcinoma, prostatitis, malacoplakia, Peyronie's disease, penile carcinoma, squamous cell hyperplasia, dysmenorrhea, ovarian adenocarcinoma, Turner's syndrome, mucopurulent cervicitis, Sertoli-leydig tumors, ovarian cancer, uterine cancer, pelvic inflammatory disease, testicular cancer, prostate cancer, Klinefelter's syndrome, Young's  
30 syndrome, premature ejaculation, diabetes mellitus, cystic fibrosis, Kartagener's syndrome, testicular atrophy, testicular feminization, anorchia, ectopic testis, epididymitis, orchitis, gonorrhea, syphilis, testicular torsion, vasitis nodosa, germ cell tumors, stromal

tumors, dysmenorrhea, retroverted uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhea, Cushing's syndrome, hydatidiform moles, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding, cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, cervical neoplasms, pseudohermaphroditism, and premenstrual syndrome.

The recitation of "Musculoskeletal" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to  
10 diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), and disorders of the immune system (e.g., as described below under "Immune Activity").

In specific embodiments, a Therapeutic protein having a "Musculoskeletal" recitation in the "Preferred Indication" column of Table 1, a fusion protein containing this  
15 Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: bone cancers (e.g., osteochondromas, benign chondromas, chondroblastoma, chondromyxoid fibromas, osteoid osteomas, giant cell tumors, multiple myeloma, osteosarcomas), Paget's Disease, rheumatoid arthritis, systemic lupus erythematosus, osteomyelitis, Lyme Disease, gout, bursitis, tendonitis, osteoporosis, osteoarthritis,  
20 muscular dystrophy, mitochondrial myopathy, cachexia, and multiple sclerosis.

The recitation of "Cardiovascular" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to  
25 diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), and disorders of the cardiovascular system (e.g., as described below under "Cardiovascular Disorders").

In specific embodiments, a Therapeutic protein having a "Cardiovascular" recitation in the "Preferred Indication" column of Table 1, a fusion protein containing this  
30 Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: myxomas, fibromas, rhabdomyomas, cardiovascular abnormalities (e.g.,

congenital heart defects, cerebral arteriovenous malformations, septal defects), heart disease (e.g., heart failure, congestive heart disease, arrhythmia, tachycardia, fibrillation, pericardial Disease, endocarditis), cardiac arrest, heart valve disease (e.g., stenosis, regurgitation, prolapse), vascular disease (e.g., hypertension, coronary artery disease, angina, aneurysm, arteriosclerosis, peripheral vascular disease), hyponatremia, hypernatremia, hypokalemia, and hyperkalemia.

The recitation of "Mixed Fetal" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders").

In specific embodiments, a Therapeutic protein having a "Mixed Fetal" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: spina bifida, hydranencephaly, neurofibromatosis, fetal alcohol syndrome, diabetes mellitus, PKU, Down's syndrome, Patau syndrome, Edwards syndrome, Turner syndrome, Apert syndrome, Carpenter syndrome, Conradi syndrome, Crouzon syndrome, cutis laxa, Cornelia de Lange syndrome, Ellis-van Creveld syndrome, Holt-Oram syndrome, Kartagener syndrome, Meckel-Gruber syndrome, Noonan syndrome, Pallister-Hall syndrome, Rubinstein-Taybi syndrome, Scimitar syndrome, Smith-Lemli-Opitz syndrome, thrombocytopenia-absent radius (TAR) syndrome, Treacher Collins syndrome, Williams syndrome, Hirschsprung's disease, Meckel's diverticulum, polycystic kidney disease, Turner's syndrome, and gonadal dysgenesis, Klippel-Feil syndrome, Ostogenesis imperfecta, muscular dystrophy, Tay-Sachs disease, Wilm's tumor, neuroblastoma, and retinoblastoma.

The recitation of "Excretory" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders") and renal disorders (e.g., as described below under "Renal Disorders").

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In specific embodiments, a Therapeutic protein having a "Excretory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: bladder cancer, prostate cancer, benign prostatic hyperplasia, bladder disorders (e.g., urinary incontinence, urinary retention, urinary obstruction, urinary tract Infections, interstitial cystitis, prostatitis, neurogenic bladder, hematuria), renal disorders (e.g., hydronephrosis, proteinuria, renal failure, pyelonephritis, urolithiasis, reflux nephropathy, and unilateral obstructive uropathy).

The recitation of "Neural/Sensory" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders") and diseases or disorders of the nervous system (e.g., as described below under "Neural Activity and Neurological Diseases").

In specific embodiments, a Therapeutic protein having a "Neural/Sensory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: brain cancer (e.g., brain stem glioma, brain tumors, central nervous system (Primary) lymphoma, central nervous system lymphoma, cerebellar astrocytoma, and cerebral astrocytoma, neurodegenerative disorders (e.g., Alzheimer's Disease, Creutzfeldt-Jakob Disease, Parkinson's Disease, and Idiopathic Presenile Dementia), encephalomyelitis, cerebral malaria, meningitis, metabolic brain diseases (e.g., phenylketonuria and pyruvate carboxylase deficiency), cerebellar ataxia, ataxia telangiectasia, and AIDS Dementia Complex, schizophrenia, attention deficit disorder, hyperactive attention deficit disorder, autism, and obsessive compulsive disorders.

The recitation of "Respiratory" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein,

and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders") and diseases or disorders of the respiratory system (e.g., as described below under "Respiratory Disorders").

5 In specific embodiments, a Therapeutic protein having a "Respiratory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: cancers of the respiratory system  
10 such as larynx cancer, pharynx cancer, trachea cancer, epiglottis cancer, lung cancer, squamous cell carcinomas, small cell (oat cell) carcinomas, large cell carcinomas, and adenocarcinomas. Allergic reactions, cystic fibrosis, sarcoidosis, histiocytosis X, infiltrative lung diseases (e.g., pulmonary fibrosis and lymphoid interstitial pneumonia), obstructive airway diseases (e.g., asthma, emphysema, chronic or acute bronchitis),  
15 occupational lung diseases (e.g., silicosis and asbestosis), pneumonia, and pleurisy.

The recitation of "Endocrine" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as  
20 described below under "Hyperproliferative Disorders") and diseases or disorders of the respiratory system (e.g., as described below under "Respiratory Disorders"), renal disorders (e.g., as described below under "Renal Disorders"), and disorders of the endocrine system (e.g., as described below under "Endocrine Disorders").

In specific embodiments, a Therapeutic protein having a "Endocrine" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a  
25 Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: cancers of endocrine tissues and organs (e.g., cancers of the hypothalamus, pituitary gland, thyroid gland, parathyroid  
30 glands, pancreas, adrenal glands, ovaries, and testes), diabetes (e.g., diabetes insipidus, type I and type II diabetes mellitus), obesity, disorders related to pituitary glands (e.g., hyperpituitarism, hypopituitarism, and pituitary dwarfism), hypothyroidism,

hyperthyroidism, goiter, reproductive disorders (e.g. male and female infertility), disorders related to adrenal glands (e.g., Addison's Disease, corticosteroid deficiency, and Cushing's Syndrome), kidney cancer (e.g., hypernephroma, transitional cell cancer, and Wilm's tumor), diabetic nephropathy, interstitial nephritis, polycystic kidney disease, glomerulonephritis (e.g., IgM mesangial proliferative glomerulonephritis and glomerulonephritis caused by autoimmune disorders; such as Goodpasture's syndrome), and nephrocalcinosis.

The recitation of "Digestive" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders") and diseases or disorders of the gastrointestinal system (e.g., as described below under "Gastrointestinal Disorders").

In specific embodiments, a Therapeutic protein having a "Digestive" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: ulcerative colitis, appendicitis, Crohn's disease, hepatitis, hepatic encephalopathy, portal hypertension, cholelithiasis, cancer of the digestive system (e.g., biliary tract cancer, stomach cancer, colon cancer, gastric cancer, pancreatic cancer, cancer of the bile duct, tumors of the colon (e.g., polyps or cancers), and cirrhosis), pancreatitis, ulcerative disease, pyloric stenosis, gastroenteritis, gastritis, gastric atrophy, benign tumors of the duodenum, distension, irritable bowel syndrome, malabsorption, congenital disorders of the small intestine, bacterial and parasitic infection, megacolon, Hirschsprung's disease, aganglionic megacolon, acquired megacolon, colitis, anorectal disorders (e.g., anal fistulas, hemorrhoids), congenital disorders of the liver (e.g., Wilson's disease, hemochromatosis, cystic fibrosis, biliary atresia, and alpha1-antitrypsin deficiency), portal hypertension, cholelithiasis, and jaundice.

The recitation of "Connective/Epithelial" in the "Preferred Indication Y" column indicates that corresponding Therapeutic protein, fusion protein containing the Therapeutic protein, and fragments and variants thereof, may be used for example, to diagnose, treat,

prevent, and/or ameliorate diseases and/or disorders relating to neoplastic diseases (e.g., as described below under "Hyperproliferative Disorders"), cellular and genetic abnormalities (e.g., as described below under "Diseases at the Cellular Level"), angiogenesis (e.g., as described below under "Anti-Angiogenesis Activity"), and or to promote or inhibit  
5 regeneration (e.g., as described below under "Regeneration"), and wound healing (e.g., as described below under "Wound Healing and Epithelial Cell Proliferation").

In specific embodiments, a Therapeutic protein having a "Connective/Epithelial" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and  
10 fragments and variants thereof, may be used for example, to diagnose, treat, prevent, and/or ameliorate a disease or disorder selected from the group consisting of: connective tissue metaplasia, mixed connective tissue disease, focal epithelial hyperplasia, epithelial metaplasia, mucoepithelial dysplasia, graft v. host disease, polymyositis, cystic hyperplasia, cerebral dysplasia, tissue hypertrophy, Alzheimer's disease,  
15 lymphoproliferative disorder, Waldenström's macroglobulinemia, Crohn's disease, pernicious anemia, idiopathic Addison's disease, glomerulonephritis, bullous pemphigoid, Sjögren's syndrome, diabetes mellitus, cystic fibrosis, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, osteoporosis, osteoarthritis, periodontal disease, wound healing, relapsing polychondritis, vasculitis, polyarteritis nodosa, Wegener's  
20 granulomatosis, cellulitis, rheumatoid arthritis, psoriatic arthritis, discoid lupus erythematosus, systemic lupus erythematosus, scleroderma, CREST syndrome, Sjögren's syndrome, polymyositis, dermatomyositis, mixed connective tissue disease, relapsing polychondritis, vasculitis, Henoch-Schönlein syndrome, erythema nodosum, polyarteritis nodosa, temporal (giant cell) arteritis, Takayasu's arteritis, Wegener's granulomatosis,  
25 Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, cellulitis, keloids, Ehler Danlos syndrome, Marfan syndrome, pseudoxanthoma elasticum, osteogenesis imperfecta, chondrodysplasias, epidermolysis bullosa, Alport syndrome, and cutis laxa.

Therapeutic Protein X	Exemplary Identifier	PCT/Patent Reference	Preferred Indication Y
HETFO52	B03768	US6066724-A	Neural/Sensory, Reproductive
HETEZ10	B03769	US6066724-A	Cancer
HLICR58	B08775	WO200052160-A1	Cancer
HMCIS41	B08776	WO200052160-A1	Cancer
HCESA34	B08891	WO200017222-A1	Cancer
HCRMZ90	B08892	WO200017222-A1	Cancer
HDPXQ54	B08893	WO200017222-A1	Immune/Hematopoietic
HETCL11	B08894	WO200017222-A1	Cancer
HFXDN34	B08895	WO200017222-A1	Neural/Sensory
HKAAV24	B08896	WO200017222-A1	Cancer
HMTBE31	B08897	WO200017222-A1	Cancer
HRADL70	B08898	WO200017222-A1	Excretory, Immune/Hematopoietic
HTXGG31	B08899	WO200017222-A1	Cancer
HWHHL34	B08900	WO200017222-A1	Cancer
HYAAV40	B08901	WO200017222-A1	Immune/Hematopoietic
HPASA81	B08902	WO200017222-A1	Digestive, Endocrine, Reproductive
HCNDA61	B08903	WO200017222-A1	Digestive, Reproductive
HTHCZ41	B08904	WO200017222-A1	Cancer
HKADJ17	B08905	WO200017222-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HMSII78	B08906	WO200017222-A1	Cancer
HCFBL76	B08907	WO200017222-A1	Cancer
HFVHR84	B08908	WO200017222-A1	Connective/Epithelial, Digestive
HIBCB67	B08909	WO200017222-A1	Cancer
HCELI29	B08910	WO200017222-A1	Cancer
HAHDZ77	B08911	WO200017222-A1	Cardiovascular, Mixed Fetal
HDHMA45	B08912	WO200017222-A1	Cardiovascular, Neural/Sensory
HELAW45	B08913	WO200017222-A1	Cardiovascular
HFIAB31	B08914	WO200017222-A1	Cancer
HLWBK05	B08915	WO200017222-A1	Cancer
HLDBX13	B08916	WO200017222-A1	Digestive
HMAGA15	B08917	WO200017222-A1	Cancer
HMWFT53	B08918	WO200017222-A1	Immune/Hematopoietic
HNFJD91	B08919	WO200017222-A1	Cardiovascular, Connective/Epithelial, Immune/Hematopoietic
HTGCM55	B08920	WO200017222-A1	Cardiovascular, Digestive, Immune/Hematopoietic
HTTEX77	B08921	WO200017222-A1	Cancer
HFXDN34	B08922	WO200017222-A1	Neural/Sensory
HDPMI18	B08923	WO200017222-A1	Cancer
HETGL41	B08924	WO200017222-A1	Cancer
HPASA81	B08925	WO200017222-A1	Digestive, Endocrine,



			Reproductive
HCNDA61	B08926	WO200017222-A1	Digestive, Reproductive
HTTEX77	B08927	WO200017222-A1	Cancer
HFXDN34	B08934	WO200017222-A1	Neural/Sensory
HETGL41	B08935	WO200017222-A1	Cancer
HPASA81	B08936	WO200017222-A1	Digestive, Endocrine, Reproductive
HCNDA61	B08940	WO200017222-A1	Digestive, Reproductive
HTTEX77	B08982	WO200017222-A1	Cancer
HAOAB14	B12301	WO200029422-A1	Digestive, Musculoskeletal
HHFBY53	B12302	WO200029422-A1	Cancer
HE2FE69	B12303	WO200029422-A1	Cancer
HNHFI33	B12305	WO200029422-A1	Immune/Hematopoietic
HAMFE15	B12306	WO200029422-A1	Cancer
HAMFE82	B12307	WO200029422-A1	Cancer
HCWEM59	B12308	WO200029422-A1	Immune/Hematopoietic
HDPGE10	B12309	WO200029422-A1	Immune/Hematopoietic
HDPGP94	B12310	WO200029422-A1	Digestive, Immune/Hematopoietic
HFPBY77	B12311	WO200029422-A1	Cancer
HFXHK32	B12312	WO200029422-A1	Neural/Sensory
HMTAK05	B12313	WO200029422-A1	Cancer
HMWDC93	B12314	WO200029422-A1	Immune/Hematopoietic
HSPBY40	B12315	WO200029422-A1	Cancer
HODDO08	B12316	WO200029422-A1	Cancer
HCFNK47	B12317	WO200029422-A1	Cancer
HE2FL70	B12318	WO200029422-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
H2MBY03	B12319	WO200029422-A1	Cancer
HACBS38	B12320	WO200029422-A1	Cancer
HAGFG51	B12321	WO200029422-A1	Neural/Sensory
HBQAB44	B12322	WO200029422-A1	Neural/Sensory, Reproductive, Respiratory
HHEMA59	B12323	WO200029422-A1	Cancer
HJBAV55	B12324	WO200029422-A1	Cancer
HLHEY02	B12325	WO200029422-A1	Endocrine, Respiratory
HSAAO94	B12326	WO200029422-A1	Cancer
HTXKP61	B12327	WO200029422-A1	Cancer
HWABC21	B12328	WO200029422-A1	Cancer
HWBDI30	B12329	WO200029422-A1	Cancer
HYBAR26	B12330	WO200029422-A1	Musculoskeletal
HAJAF57	B12331	WO200029422-A1	Cancer
HAMFE15	B12332	WO200029422-A1	Cancer
HAMFE82	B12333	WO200029422-A1	Cancer
HAMFE15	B12338	WO200029422-A1	Cancer
HAMFE82	B12339	WO200029422-A1	Cancer
HLDOK36	B15551	WO200056752-A2	Cancer
HDPBW68	B15552	WO200056752-A2	Cancer
HHEFO24	B15553	WO200056752-A2	Cardiovascular,

			Immune/Hematopoietic, Neural/Sensory
HEGAL46	B15554	WO200056752-A2	Cancer
HFOYC02	B15555	WO200056752-A2	Cancer
HDABV82	B15556	WO200056752-A2	Cancer
HCEMU42	B15557	WO200056752-A2	Cancer
HSIFO61	B15558	WO200056752-A2	Cancer
HDPBW68	B15559	WO200056752-A2	Cancer
HDPBW68	B15562	WO200056752-A2	Cancer
HSIFO61	B15566	WO200056752-A2	Cancer
HOEAL47	B18715	WO200054651-A2	Cancer
HE9SF68	B18755	WO200055204-A1	Cancer
HLICQ90	B24437	WO200035937-A1	Cancer
HNTSM04	B24438	WO200035937-A1	Cancer
HMCAL59	B24439	WO200035937-A1	Cancer
HMACO04	B24440	WO200035937-A1	Cancer
HMAHY59	B24441	WO200035937-A1	Cancer
HFXLL52	B24442	WO200035937-A1	Neural/Sensory
HKABY55	B24443	WO200035937-A1	Cancer
HCQCF36	B24444	WO200035937-A1	Digestive, Immune/Hematopoietic
HTADO22	B24445	WO200035937-A1	Immune/Hematopoietic
HHFHD92	B24446	WO200035937-A1	Cancer
HNGFW58	B24447	WO200035937-A1	Cancer
HOEFV61	B24448	WO200035937-A1	Cancer
HPIAQ68	B24449	WO200035937-A1	Immune/Hematopoietic, Reproductive
HNFFY60	B24450	WO200035937-A1	Cancer
HCE3H20	B24451	WO200035937-A1	Cancer
HE8EW79	B24452	WO200035937-A1	Cancer
HTTDF41	B24453	WO200035937-A1	Cancer
HSSGJ45	B24454	WO200035937-A1	Cancer
HLWBY76	B24455	WO200035937-A1	Cancer
HDPBN34	B24456	WO200035937-A1	Immune/Hematopoietic
HMSHY73	B24457	WO200035937-A1	Cancer
HPRBF19	B24458	WO200035937-A1	Cancer
HNFJE06	B24459	WO200035937-A1	Immune/Hematopoietic, Musculoskeletal
HCHCF61	B24460	WO200035937-A1	Reproductive
HBJLH40	B24461	WO200035937-A1	Cancer
HDPMV72	B24462	WO200035937-A1	Cancer
HEMFA84	B24463	WO200035937-A1	Cancer
HTOHW95	B24464	WO200035937-A1	Cancer
HUNAH63	B24465	WO200035937-A1	Reproductive
HISBT59	B24466	WO200035937-A1	Cancer
HNTAS52	B24467	WO200035937-A1	Cancer
HRACM44	B24468	WO200035937-A1	Excretory, Immune/Hematopoietic
HFPE577	B24469	WO200035937-A1	Cancer
HUSXU29	B24470	WO200035937-A1	Cancer
HOHBB49	B24471	WO200035937-A1	Musculoskeletal
HRABX31	B24472	WO200035937-A1	Excretory, Immune/Hematopoietic, Musculoskeletal
HROBD68	B24473	WO200035937-A1	Cancer
HMHBE18	B24474	WO200035937-A1	Cancer

HNHDY21	B24475	WO200035937-A1	Immune/Hematopoietic
HOEBZ89	B24476	WO200035937-A1	Cancer
HYAAJ71	B24477	WO200035937-A1	Immune/Hematopoietic
HTEKS16	B24478	WO200035937-A1	Connective/Epithelial, Mixed Fetal, Reproductive
HCUFX40	B24479	WO200035937-A1	Immune/Hematopoietic
HCWDL75	B24480	WO200035937-A1	Cardiovascular, Immune/Hematopoietic
HNHKJ57	B24481	WO200035937-A1	Immune/Hematopoietic
HCMSS06	B24482	WO200035937-A1	Cancer
HIBCE35	B24483	WO200035937-A1	Cancer
HE8EW79	B24484	WO200035937-A1	Cancer
HTTDF41	B24485	WO200035937-A1	Cancer
HSSGJ45	B24486	WO200035937-A1	Cancer
HCMSS06	B24487	WO200035937-A1	Cancer
HCMSS06	B24597	WO200035937-A1	Cancer
HAOAB64	B25576	WO200029435-A1	Musculoskeletal, Reproductive
HOHCH55	B25577	WO200029435-A1	Cancer
HTLEW81	B25578	WO200029435-A1	Cancer
HARAO44	B25579	WO200029435-A1	Neural/Sensory
HDPCL05	B25580	WO200029435-A1	Immune/Hematopoietic
HDP UW68	B25581	WO200029435-A1	Cancer
HOHBY69	B25582	WO200029435-A1	Cancer
HCDDP40	B25583	WO200029435-A1	Immune/Hematopoietic, Musculoskeletal
HUSAQ05	B25585	WO200029435-A1	Cancer
HOUDJ81	B25586	WO200029435-A1	Cancer
HPWCM76	B25587	WO200029435-A1	Reproductive
HOHCH55	B25588	WO200029435-A1	Cancer
HDPCL05	B25589	WO200029435-A1	Immune/Hematopoietic
HOHBY69	B25590	WO200029435-A1	Cancer
HUSAQ05	B25592	WO200029435-A1	Cancer
HOUDJ81	B25593	WO200029435-A1	Cancer
HUSAQ05	B25618	WO200029435-A1	Cancer
HE8NG02	B25665	WO200043495-A2	Mixed Fetal, Reproductive
HWBDM37	B25666	WO200043495-A2	Digestive, Immune/Hematopoietic, Reproductive
HODFN71	B25668	WO200043495-A2	Mixed Fetal, Reproductive
HODGE68	B25669	WO200043495-A2	Reproductive
HCECN54	B25670	WO200043495-A2	Excretory, Neural/Sensory
HE8UB86	B25671	WO200043495-A2	Cancer
HNH KY10	B25672	WO200043495-A2	Immune/Hematopoietic
HNHLB93	B25673	WO200043495-A2	Immune/Hematopoietic
HNHON23	B25674	WO200043495-A2	Immune/Hematopoietic, Musculoskeletal
HTEPG70	B25675	WO200043495-A2	Reproductive
HNHOJ75	B25676	WO200043495-A2	Immune/Hematopoietic
HDTIT10	B25677	WO200043495-A2	Cancer
HKAOS84	B25678	WO200043495-A2	Connective/Epithelial
HAPUC89	B25679	WO200043495-A2	Cancer

HWAAD63	B25680	WO200043495-A2	Endocrine, Excretory, Immune/Hematopoietic
HUCPD31	B25681	WO200043495-A2	Cancer
HDQHD03	B25682	WO200043495-A2	Immune/Hematopoietic, Neural/Sensory
HKAKK09	B25683	WO200043495-A2	Connective/Epithelial, Digestive, Mixed Fetal
HOCNF19	B25684	WO200043495-A2	Digestive
HTLIT32	B25685	WO200043495-A2	Reproductive
HODEJ32	B25686	WO200043495-A2	Reproductive
HNHMOV54	B25687	WO200043495-A2	Immune/Hematopoietic
HODEE95	B25688	WO200043495-A2	Reproductive
HLHAM10	B25689	WO200043495-A2	Cancer
HNHOG73	B25690	WO200043495-A2	Immune/Hematopoietic
HGBNM47	B25691	WO200043495-A2	Cancer
HAUBA08	B25692	WO200043495-A2	Cancer
HYBBE75	B25693	WO200043495-A2	Musculoskeletal
HTLGY87	B25694	WO200043495-A2	Cancer
HNHPD10	B25695	WO200043495-A2	Immune/Hematopoietic
HODEI83	B25696	WO200043495-A2	Reproductive
HMUAI20	B25697	WO200043495-A2	Cancer
HE9OW20	B25698	WO200043495-A2	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HDTIT10	B25699	WO200043495-A2	Cancer
HWAAD63	B25700	WO200043495-A2	Endocrine, Excretory, Immune/Hematopoietic
HWAAD63	B25701	WO200043495-A2	Endocrine, Excretory, Immune/Hematopoietic
HEMCV19	B25703	WO200043495-A2	Cancer
HEMCV19	B25704	WO200043495-A2	Cancer
HEMCV19	B25705	WO200043495-A2	Cancer
HAUBA08	B25706	WO200043495-A2	Cancer
HEMCV19	B25707	WO200043495-A2	Cancer
HE9OW20	B25715	WO200043495-A2	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HT4SB02	B27560	WO200055175-A1	Immune/Hematopoietic
HCHAC68	B27562	WO200055175-A1	Cancer
HCHCA79	B27563	WO200055175-A1	Digestive, Neural/Sensory, Reproductive
HCHMY57	B27564	WO200055175-A1	Cancer
HCHOY52	B27566	WO200055175-A1	Cancer
HCHQB93	B27567	WO200055175-A1	Cancer
HCMSA37	B27568	WO200055175-A1	Cardiovascular
HCMSX51	B27570	WO200055175-A1	Cancer
HCHAI74	B27571	WO200055175-A1	Digestive
HCPAE41	B27578	WO200055175-A1	Cancer
HCQAQ47	B27580	WO200055175-A1	Cancer
HCQBH72	B27581	WO200055175-A1	Digestive, Excretory,

			Immune/Hematopoietic
HCQDD32	B27585	WO200055175-A1	Digestive, Immune/Hematopoietic, Reproductive
HCQDT67	B27586	WO200055175-A1	Cancer
HCRA Y10	B27587	WO200055175-A1	Cancer
HCRBI79	B27589	WO200055175-A1	Cancer
HN FAD50	B27591	WO200055175-A1	Cancer
HCRNF78	B27592	WO200055175-A1	Cancer
HCUAF85	B27594	WO200055175-A1	Immune/Hematopoietic
HCUBM41	B27598	WO200055175-A1	Immune/Hematopoietic
HCUBN69	B27599	WO200055175-A1	Immune/Hematopoietic
HCUDD64	B27602	WO200055175-A1	Cancer
HCUEC55	B27604	WO200055175-A1	Immune/Hematopoietic
HCUFC77	B27607	WO200055175-A1	Cancer
HB JBR40	B27686	WO200055201-A1	Immune/Hematopoietic
HB JCH46	B27687	WO200055201-A1	Immune/Hematopoietic, Musculoskeletal
HB JFU30	B27698	WO200055201-A1	Cancer
HB JAY14	B27704	WO200055201-A1	Immune/Hematopoietic
HB JND04	B27708	WO200055201-A1	Cancer
HB KEA94	B27711	WO200055201-A1	Cancer
HB JDS79	B27712	WO200055201-A1	Cancer
HB KEI41	B27713	WO200055201-A1	Endocrine, Mixed Fetal, Reproductive
HB JHO83	B27720	WO200055201-A1	Immune/Hematopoietic, Reproductive
HBMCT40	B27721	WO200055201-A1	Cancer
HB MTX26	B27724	WO200055201-A1	Immune/Hematopoietic
HB MTY48	B27725	WO200055201-A1	Immune/Hematopoietic, Reproductive
HBMUD59	B27726	WO200055201-A1	Cancer
HBMUI10	B27727	WO200055201-A1	Cancer
HCEEU18	B27794	WO200055199-A1	Cancer
HCDCB03	B27795	WO200055199-A1	Cancer
HCE1G78	B27797	WO200055199-A1	Cancer
HCDEB19	B27799	WO200055199-A1	Cancer
HCEDR26	B27801	WO200055199-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HCDBW67	B27804	WO200055199-A1	Cancer
HCDDX81	B27808	WO200055199-A1	Musculoskeletal
HB ZAI75	B27809	WO200055199-A1	Digestive, Reproductive
HC DEN46	B27810	WO200055199-A1	Cancer
HCE1D45	B27811	WO200055199-A1	Cancer
HCE1Y27	B27813	WO200055199-A1	Digestive, Neural/Sensory, Reproductive
HCE2I23	B27816	WO200055199-A1	Neural/Sensory
HCE2P90	B27817	WO200055199-A1	Neural/Sensory
HCE3A54	B27818	WO200055199-A1	Neural/Sensory
HCE3D89	B27819	WO200055199-A1	Endocrine, Neural/Sensory
HCE3N23	B27820	WO200055199-A1	Cancer

HCE4T64	B27821	WO200055199-A1	Cancer
HCE5J64	B27823	WO200055199-A1	Digestive, Neural/Sensory
HCECO77	B27824	WO200055199-A1	Cancer
HCEDH42	B27825	WO200055199-A1	Neural/Sensory
HCEDJ05	B27826	WO200055199-A1	Neural/Sensory
HCEEE79	B27829	WO200055199-A1	Neural/Sensory
HCEFH31	B27837	WO200055199-A1	Cancer
HCDDL48	B27838	WO200055199-A1	Musculoskeletal
HFVIC33	B27908	WO200055171-A1	Cancer
HEMAH05	B27909	WO200055171-A1	Cancer
HHSBI65	B27911	WO200055171-A1	Cancer
HLEAA24	B27917	WO200055171-A1	Immune/Hematopoietic
HPTTQ91	B27919	WO200055171-A1	Cancer
HPMGY89	B27923	WO200055171-A1	Cancer
H2LAO03	B27933	WO200055171-A1	Cancer
H2MBA76	B27937	WO200055171-A1	Cancer
H2MBF60	B27938	WO200055171-A1	Cancer
H6BSF56	B27939	WO200055171-A1	Cancer
H6BSM88	B27940	WO200055171-A1	Cancer
H6EEU40	B27941	WO200055171-A1	Cancer
HACAB68	B27943	WO200055171-A1	Connective/Epithelial, Immune/Hematopoietic
HACBA04	B27945	WO200055171-A1	Cancer
HACBJ11	B27946	WO200055171-A1	Cancer
HACBS86	B27947	WO200055171-A1	Cancer
HACBT91	B27948	WO200055171-A1	Cancer
HADAB60	B27951	WO200055171-A1	Cancer
HADDE71	B27956	WO200055171-A1	Cancer
HAGFU31	B28014	WO200055177-A2	Neural/Sensory
HAPBR13	B28017	WO200055177-A2	Cancer
HAQBG57	B28020	WO200055177-A2	Cancer
HARAE26	B28022	WO200055177-A2	Neural/Sensory
HAHEM51	B28032	WO200055177-A2	Cardiovascular
HAICL90	B28035	WO200055177-A2	Digestive, Immune/Hematopoietic, Reproductive
HAMFC67	B28038	WO200055177-A2	Cancer
HAPBU09	B28041	WO200055177-A2	Cancer
HAPNL62	B28043	WO200055177-A2	Cancer
HAPNY10	B28045	WO200055177-A2	Cancer
HAPQU71	B28048	WO200055177-A2	Cancer
HARAT69	B28054	WO200055177-A2	Cancer
HAGEU26	B28059	WO200055177-A2	Neural/Sensory
HANGB24	B28060	WO200055177-A2	Cancer
HNGER85	B28286	WO200058355-A1	Immune/Hematopoietic
HNGET33	B28287	WO200058355-A1	Immune/Hematopoietic
HNGFA25	B28292	WO200058355-A1	Immune/Hematopoietic
HNGFG04	B28297	WO200058355-A1	Immune/Hematopoietic
HNGFG74	B28298	WO200058355-A1	Immune/Hematopoietic
HNGFI21	B28301	WO200058355-A1	Cancer
HNGFM31	B28302	WO200058355-A1	Immune/Hematopoietic
HCUCK44	B28303	WO200058355-A1	Cancer
HNGFQ18	B28304	WO200058355-A1	Immune/Hematopoietic
HNGFR54	B28305	WO200058355-A1	Immune/Hematopoietic

HNGGF13	B28309	WO200058355-A1	Immune/Hematopoietic
HNGGL11	B28311	WO200058355-A1	Immune/Hematopoietic
HNGGP65	B28312	WO200058355-A1	Immune/Hematopoietic
HNGHM47	B28316	WO200058355-A1	Immune/Hematopoietic
HNGIH40	B28318	WO200058355-A1	Immune/Hematopoietic
HNGIM83	B28321	WO200058355-A1	Immune/Hematopoietic
HNGIS27	B28322	WO200058355-A1	Immune/Hematopoietic
HADFB84	B28707	WO200055198-A1	Cancer
HADFD01	B28708	WO200055198-A1	Cancer
HADFK11	B28709	WO200055198-A1	Connective/Epithelial
HADFT44	B28710	WO200055198-A1	Connective/Epithelial, Mixed Fetal, Neural/Sensory
HADGD93	B28714	WO200055198-A1	Cardiovascular, Connective/Epithelial
HAFBB15	B28716	WO200055198-A1	Cancer
HAGAB62	B28718	WO200055198-A1	Cancer
HAGAF75	B28720	WO200055198-A1	Digestive, Neural/Sensory
HAGAZ36	B28722	WO200055198-A1	Neural/Sensory
H2CBH91	B28725	WO200055198-A1	Cancer
HAGBV29	B28730	WO200055198-A1	Immune/Hematopoietic, Neural/Sensory
HAGCH67	B28732	WO200055198-A1	Neural/Sensory
HAGCI69	B28733	WO200055198-A1	Neural/Sensory, Reproductive
HAGCT33	B28734	WO200055198-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HAGDG84	B28737	WO200055198-A1	Immune/Hematopoietic, Neural/Sensory
HAGDO70	B28743	WO200055198-A1	Cancer
HAGDW68	B28746	WO200055198-A1	Endocrine, Neural/Sensory
HAGEG10	B28748	WO200055198-A1	Cancer
HAGEK37	B28750	WO200055198-A1	Cancer
HTADO61	B29803	WO200061779-A1	Cancer
HELDH39	B29805	WO200061779-A1	Cancer
HSLCV16	B29806	WO200061779-A1	Cancer
HTOJL95	B29807	WO200061779-A1	Cancer
HOEBK60	B29810	WO200061779-A1	Cancer
HSAWN53	B29813	WO200061779-A1	Immune/Hematopoietic
HORBS82	B29814	WO200061779-A1	Cancer
HORBV76	B29815	WO200061779-A1	Cardiovascular, Immune/Hematopoietic, Reproductive
HTEHA56	B29822	WO200061779-A1	Cancer
HMWER46	B29828	WO200061779-A1	Cancer
HPRAD30	B29829	WO200061779-A1	Cancer
HTLDD89	B29832	WO200061779-A1	Reproductive
HMCJC19	B29835	WO200061779-A1	Immune/Hematopoietic
HROBW46	B29841	WO200061779-A1	Digestive, Immune/Hematopoietic
HSOBB94	B29842	WO200061779-A1	Cancer
HSOBP75	B29844	WO200061779-A1	Cancer
HNHKS19	B29848	WO200061779-A1	Immune/Hematopoietic,

[REDACTED]



			Reproductive
HFIDS78	B32399	WO200047602-A1	Connective/Epithelial, Digestive, Musculoskeletal
HSDIB20	B32400	WO200047602-A1	Cancer
HHEPU04	B32401	WO200047602-A1	Cancer
HNGKN89	B32402	WO200047602-A1	Immune/Hematopoietic
HE9TH18	B32403	WO200047602-A1	Cancer
HHFHM89	B32404	WO200047602-A1	Cancer
HASAV70	B32405	WO200047602-A1	Cancer
HSDFJ26	B32406	WO200047602-A1	Cancer
HODAA16	B32407	WO200047602-A1	Cancer
HODAA16	B32408	WO200047602-A1	Cancer
HGBIB74	B32409	WO200047602-A1	Cancer
HCRMU04	B32410	WO200047602-A1	Cancer
HSAAO65	B32411	WO200047602-A1	Cancer
HSAAO65	B32412	WO200047602-A1	Cancer
HSMBB92	B32413	WO200047602-A1	Cancer
HHEPU04	B32414	WO200047602-A1	Cancer
HLDNA86	B32415	WO200047602-A1	Cancer
HE9TH18	B32416	WO200047602-A1	Cancer
HHFHM33	B32447	WO200047602-A1	Cancer
HUUAV63	B32481	WO200047602-A1	Cancer
HE2CJ53	B33721	WO200056753-A1	Cancer
HE2HF76	B33724	WO200056753-A1	Cancer
HDTDE66	B33729	WO200056753-A1	Cancer
HDTKJ29	B33735	WO200056753-A1	Cancer
HDTLM18	B33736	WO200056753-A1	Immune/Hematopoietic
HE2AI94	B33740	WO200056753-A1	Cancer
HE2BD72	B33741	WO200056753-A1	Cancer
HE2CH58	B33744	WO200056753-A1	Digestive, Mixed Fetal
HE2NW57	B33753	WO200056753-A1	Mixed Fetal
HE2PJ56	B33755	WO200056753-A1	Cancer
HE2PO93	B33756	WO200056753-A1	Cancer
HE6AU52	B33757	WO200056753-A1	Mixed Fetal
HAEAV42	B33758	WO200056753-A1	Cancer
HE2AT61	B33759	WO200056753-A1	Cancer
HE2CK47	B33761	WO200056753-A1	Cancer
HE2DJ84	B33763	WO200056753-A1	Cancer
HE2CJ53	B33770	WO200056753-A1	Cancer
HSHAS72	B33832	WO200056753-A1	Cancer
HEMDR05	B33845	WO200056881-A1	Cardiovascular, Digestive, Immune/Hematopoietic
HADXA10	B33846	WO200056881-A1	Cancer
HEOMF59	B33847	WO200056881-A1	Immune/Hematopoietic
HEONP08	B33854	WO200056881-A1	Immune/Hematopoietic
HEPAD15	B33855	WO200056881-A1	Endocrine, Reproductive
HEIAC52	B33860	WO200056881-A1	Cancer
HEQAP92	B33862	WO200056881-A1	Cancer
HEQBM94	B33865	WO200056881-A1	Cancer
HETAA62	B33870	WO200056881-A1	Cancer
HETAY67	B33873	WO200056881-A1	Connective/Epithelial, Reproductive

HETDP76	B33874	WO200056881-A1	Cancer
HEQBF89	B33875	WO200056881-A1	Reproductive
HETIN36	B33877	WO200056881-A1	Cancer
HFAUA23	B33881	WO200056881-A1	Cancer
HFCAG75	B33882	WO200056881-A1	Cancer
HFCAQ17	B33883	WO200056881-A1	Cancer
HFCDN13	B33887	WO200056881-A1	Cancer
HFCDT67	B33888	WO200056881-A1	Cancer
HFCEI04	B33891	WO200056881-A1	Neural/Sensory
HTSGY89	B33946	WO200056881-A1	Cancer
HFCAQ17	B33947	WO200056881-A1	Cancer
HGLAH86	B33965	WO200056765-A1	Immune/Hematopoietic
HHEBP28	B33971	WO200056765-A1	Cancer
HHEMC55	B33973	WO200056765-A1	Immune/Hematopoietic
HHEMM20	B33974	WO200056765-A1	Immune/Hematopoietic
HHEMP35	B33976	WO200056765-A1	Cancer
HHEMZ08	B33977	WO200056765-A1	Cancer
HHENR74	B33980	WO200056765-A1	Immune/Hematopoietic
HHEOK77	B33983	WO200056765-A1	Cancer
HHEQI04	B33986	WO200056765-A1	Connective/Epithelial, Excretory, Immune/Hematopoietic
HHFBA31	B33987	WO200056765-A1	Cancer
HHFFF87	B33992	WO200056765-A1	Cancer
HHFFL34	B33993	WO200056765-A1	Cancer
HHFFS40	B33994	WO200056765-A1	Cancer
HHGBF91	B34005	WO200056765-A1	Cancer
HE9NB82	B34092	WO200056755-A1	Cancer
HEAAC21	B34095	WO200056755-A1	Cancer
HEAAM54	B34100	WO200056755-A1	Reproductive
HEAAU28	B34102	WO200056755-A1	Reproductive
HEBAT05	B34104	WO200056755-A1	Cancer
HEBCN80	B34107	WO200056755-A1	Neural/Sensory
HEBCY54	B34108	WO200056755-A1	Cancer
HEBDW31	B34111	WO200056755-A1	Cancer
HEBFL36	B34112	WO200056755-A1	Neural/Sensory
HEBGE07	B34114	WO200056755-A1	Neural/Sensory
HEBGE23	B34115	WO200056755-A1	Cancer
HEGAI20	B34119	WO200056755-A1	Reproductive
HEBCI18	B34121	WO200056755-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HEBDF90	B34125	WO200056755-A1	Cancer
HELDK79	B34127	WO200056755-A1	Cardiovascular
HELEL76	B34130	WO200056755-A1	Cancer
HELFO30	B34131	WO200056755-A1	Cancer
HEMCJ80	B34138	WO200056755-A1	Cancer
HDPAR73	B34201	WO200056755-A1	Cancer
HDPAR73	B34202	WO200056755-A1	Cancer
HATBI94	B34222	WO200055352-A2	Cancer
HATCB45	B34224	WO200055352-A2	Endocrine, Immune/Hematopoietic
HATDW05	B34229	WO200055352-A2	Endocrine
HATEH20	B34231	WO200055352-A2	Cancer
HAWBA65	B34233	WO200055352-A2	Cancer

HBBBA42	B34236	WO200055352-A2	Cancer
HBBBE83	B34238	WO200055352-A2	Cancer
HBBMA11	B34239	WO200055352-A2	Neural/Sensory
HBCGE46	B34244	WO200055352-A2	Musculoskeletal
HBGML95	B34249	WO200055352-A2	Reproductive
HBHAA05	B34251	WO200055352-A2	Neural/Sensory
HBHAA53	B34252	WO200055352-A2	Neural/Sensory
HBIAA59	B34253	WO200055352-A2	Cancer
HBICW51	B34262	WO200055352-A2	Digestive, Immune/Hematopoietic, Neural/Sensory
HFCET43	B34299	WO200056883-A1	Cancer
HFEAG55	B34302	WO200056883-A1	Cancer
HFEAY59	B34304	WO200056883-A1	Connective/Epithelial
HFFAV61	B34308	WO200056883-A1	Neural/Sensory
HFGAN63	B34312	WO200056883-A1	Cancer
HFICH70	B34316	WO200056883-A1	Musculoskeletal
HFIHQ57	B34317	WO200056883-A1	Musculoskeletal, Reproductive
HFIHZ75	B34318	WO200056883-A1	Cancer
HFIIA29	B34321	WO200056883-A1	Cancer
HFIJD81	B34322	WO200056883-A1	Cancer
HFIUK66	B34324	WO200056883-A1	Cancer
HFIXC39	B34326	WO200056883-A1	Cancer
HFIXC69	B34327	WO200056883-A1	Cancer
HLWAU42	B34329	WO200056883-A1	Cancer
HFIZF51	B34331	WO200056883-A1	Musculoskeletal
HFKDX53	B34333	WO200056883-A1	Cancer
HFKEG63	B34335	WO200056883-A1	Excretory
HFKES05	B34336	WO200056883-A1	Cancer
HFKES35	B34337	WO200056883-A1	Cancer
HFKEU12	B34338	WO200056883-A1	Excretory
HFOYR54	B34344	WO200056883-A1	Cancer
HFPBJ64	B34347	WO200056883-A1	Musculoskeletal, Neural/Sensory
HFXBV67	B34441	WO200056767-A1	Digestive, Neural/Sensory
HFXBY20	B34442	WO200056767-A1	Neural/Sensory
HFXGT51	B34462	WO200056767-A1	Neural/Sensory
HFXGW04	B34463	WO200056767-A1	Cancer
HFXHL83	B34466	WO200056767-A1	Neural/Sensory
HFXJB21	B34468	WO200056767-A1	Neural/Sensory
HFXJN93	B34469	WO200056767-A1	Neural/Sensory
HFXJT53	B34470	WO200056767-A1	Cancer
HFXLK91	B34472	WO200056767-A1	Cancer
HFXHM49	B34473	WO200056767-A1	Neural/Sensory
HGBDV35	B34474	WO200056767-A1	Cancer
HEPCU48	B34476	WO200056767-A1	Cancer
HGBGN34	B34478	WO200056767-A1	Connective/Epithelial, Digestive, Reproductive
HGBGX31	B34479	WO200056767-A1	Cancer
HGBHP91	B34482	WO200056767-A1	Digestive
HCEFN51	B34580	WO200056751-A1	Cancer
HCEFZ82	B34581	WO200056751-A1	Cancer
HCEJL08	B34585	WO200056751-A1	Cancer

HCENN67	B34588	WO200056751-A1	Digestive, Endocrine, Neural/Sensory
HCEPC90	B34592	WO200056751-A1	Neural/Sensory
HCETL19	B34597	WO200056751-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HCFAT42	B34602	WO200056751-A1	Immune/Hematopoietic
HCFAT66	B34603	WO200056751-A1	Immune/Hematopoietic
HCFBM77	B34605	WO200056751-A1	Immune/Hematopoietic
HCFLJ52	B34611	WO200056751-A1	Cancer
HCFLP48	B34613	WO200056751-A1	Immune/Hematopoietic
HCFLQ12	B34614	WO200056751-A1	Cancer
HCFMA39	B34617	WO200056751-A1	Immune/Hematopoietic
HCFML07	B34619	WO200056751-A1	Cancer
HCFMX88	B34620	WO200056751-A1	Immune/Hematopoietic, Neural/Sensory
HCFNN16	B34623	WO200056751-A1	Cancer
HCFNN75	B34624	WO200056751-A1	Cancer
HLYBI48	B34774	WO200058356-A1	Immune/Hematopoietic
HFKE535	B34777	WO200058356-A1	Cancer
HLYBU15	B34781	WO200058356-A1	Immune/Hematopoietic
HLYDX01	B34789	WO200058356-A1	Cancer
HLYEA60	B34790	WO200058356-A1	Cancer
HLYEU59	B34793	WO200058356-A1	Immune/Hematopoietic
HLYGE16	B34794	WO200058356-A1	Cancer
HLYGV19	B34795	WO200058356-A1	Cancer
HLYGY91	B34796	WO200058356-A1	Cancer
HMADJ14	B34797	WO200058356-A1	Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HMAGF01	B34798	WO200058356-A1	Cancer
HMDAB44	B34806	WO200058356-A1	Neural/Sensory
HMDAE88	B34808	WO200058356-A1	Neural/Sensory
HMDAG62	B34809	WO200058356-A1	Cancer
HMDAK20	B34810	WO200058356-A1	Neural/Sensory
HMECM77	B34815	WO200058356-A1	Cardiovascular
HMECQ59	B34816	WO200058356-A1	Cancer
HMEEZ07	B34821	WO200058356-A1	Cardiovascular, Reproductive
HDPCV29	B34856	WO200056766-A1	Immune/Hematopoietic
HDPCW16	B34857	WO200056766-A1	Cancer
HDPFG13	B34859	WO200056766-A1	Cancer
HDPFU43	B34860	WO200056766-A1	Cancer
HDPFZ05	B34861	WO200056766-A1	Immune/Hematopoietic, Neural/Sensory
HDPGA84	B34862	WO200056766-A1	Cancer
HDPIH25	B34864	WO200056766-A1	Cancer
HDPKC55	B34866	WO200056766-A1	Cardiovascular, Immune/Hematopoietic, Reproductive
HDPNC21	B34868	WO200056766-A1	Cancer
HDPOL37	B34869	WO200056766-A1	Immune/Hematopoietic, Reproductive
HDPSZ07	B34871	WO200056766-A1	Immune/Hematopoietic
HDPXN20	B34872	WO200056766-A1	Immune/Hematopoietic

HDQGN08	B34874	WO200056766-A1	Immune/Hematopoietic
HDPGE24	B34877	WO200056766-A1	Cancer
HDPJV53	B34878	WO200056766-A1	Immune/Hematopoietic
HDPOC24	B34881	WO200056766-A1	Cancer
HDPPC19	B34883	WO200056766-A1	Immune/Hematopoietic
HBCAO31	B34886	WO200056766-A1	Cancer
HDQGD06	B34889	WO200056766-A1	Cancer
HDRAA17	B34890	WO200056766-A1	Cancer
HDRAC68	B34891	WO200056766-A1	Cancer
HDSAH37	B34893	WO200056766-A1	Connective/Epithelial
HDSAP15	B34896	WO200056766-A1	Cancer
HDTAS57	B34897	WO200056766-A1	Cancer
HDPFU43	B34916	WO200056766-A1	Cancer
HDPKC55	B34932	WO200056766-A1	Cardiovascular, Immune/Hematopoietic, Reproductive
HPRCB54	B36696	WO200071150-A1	Cancer
HMQAT69	B37348	WO200058335-A1	Cancer
HMQBL90	B37349	WO200058335-A1	Digestive, Immune/Hematopoietic
HMQCX41	B37354	WO200058335-A1	Immune/Hematopoietic
HMQDU07	B37356	WO200058335-A1	Digestive, Immune/Hematopoietic, Musculoskeletal
HMSDI67	B37365	WO200058335-A1	Digestive, Immune/Hematopoietic
HMSHC86	B37372	WO200058335-A1	Immune/Hematopoietic
HMSII36	B37376	WO200058335-A1	Immune/Hematopoietic
HMSIT42	B37377	WO200058335-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HMSKQ91	B37381	WO200058335-A1	Immune/Hematopoietic
HMTAT36	B37384	WO200058335-A1	Cancer
HMUBK53	B37390	WO200058335-A1	Cancer
HMUBO15	B37392	WO200058335-A1	Cancer
HMUBZ15	B37393	WO200058335-A1	Cancer
HMSLF15	B37984	WO200055371-A1	Cancer
HKAET41	B37985	WO200055371-A1	Connective/Epithelial, Digestive, Reproductive
HE9RJ42	B37987	WO200055371-A1	Mixed Fetal
HDPAS92	B37988	WO200055371-A1	Cancer
HATDF29	B37989	WO200055371-A1	Cancer
HWLHH15	B37990	WO200055371-A1	Digestive
HBXFL29	B37991	WO200055371-A1	Cancer
HKGBF67	B37992	WO200055371-A1	Cancer
HWHGP71	B37993	WO200055371-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HLWCU38	B37994	WO200055371-A1	Cancer
HMTAX46	B37995	WO200055371-A1	Cancer
HIBEU15	B37996	WO200055371-A1	Excretory, Immune/Hematopoietic, Neural/Sensory
HDPQV66	B37997	WO200055371-A1	Cancer
HFXGW52	B37998	WO200055371-A1	Neural/Sensory

HHEQR55	B37999	WO200055371-A1	Immune/Hematopoietic
HNHNW84	B38000	WO200055371-A1	Immune/Hematopoietic
HABX74	B38001	WO200055371-A1	Cancer
HCUGE72	B38002	WO200055371-A1	Cancer
HTEQI22	B38003	WO200055371-A1	Cancer
HDPYE41	B38004	WO200055371-A1	Immune/Hematopoietic
HDTII23	B38005	WO200055371-A1	Immune/Hematopoietic
HAMFL84	B38007	WO200055371-A1	Cancer
HTELW37	B38008	WO200055371-A1	Reproductive
HNGOU56	B38009	WO200055371-A1	Immune/Hematopoietic
HOUHD63	B38010	WO200055371-A1	Cancer
HPJCX13	B38011	WO200055371-A1	Cancer
HNHCT15	B38012	WO200055371-A1	Cancer
HKGBF67	B38013	WO200055371-A1	Cancer
HWHGP71	B38014	WO200055371-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HTEQI22	B38016	WO200055371-A1	Cancer
HJBCI01	B38017	WO200055371-A1	Cancer
HTSFV18	B38018	WO200055371-A1	Cancer
HPJBF63	B38019	WO200055371-A1	Cancer
HWHGP71	B38044	WO200055371-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HNEDU46	B38119	WO200058468-A2	Cancer
HNFDY31	B38124	WO200058468-A2	Cancer
HNFEA17	B38125	WO200058468-A2	Cancer
HNFET12	B38127	WO200058468-A2	Immune/Hematopoietic
HNFGRO8	B38129	WO200058468-A2	Immune/Hematopoietic
HNFGW37	B38130	WO200058468-A2	Immune/Hematopoietic
HNFGW53	B38131	WO200058468-A2	Cancer
HNFHA34	B38132	WO200058468-A2	Cancer
HNFEJ27	B38137	WO200058468-A2	Immune/Hematopoietic
HNGAM58	B38143	WO200058468-A2	Immune/Hematopoietic
HNGAT83	B38144	WO200058468-A2	Immune/Hematopoietic
HNGBE63	B38148	WO200058468-A2	Immune/Hematopoietic
HNGBH53	B38149	WO200058468-A2	Immune/Hematopoietic
HNGBJ74	B38150	WO200058468-A2	Immune/Hematopoietic
HNGBQ61	B38152	WO200058468-A2	Immune/Hematopoietic
HNGBW25	B38154	WO200058468-A2	Immune/Hematopoietic
HNGCF64	B38156	WO200058468-A2	Immune/Hematopoietic
HNGDH22	B38158	WO200058468-A2	Immune/Hematopoietic
HNGDQ38	B38161	WO200058468-A2	Immune/Hematopoietic
HNGDR39	B38162	WO200058468-A2	Immune/Hematopoietic
HNGEA34	B38165	WO200058468-A2	Digestive, Immune/Hematopoietic
HTOJB02	B38205	WO200058469-A1	Immune/Hematopoietic
HSDEA26	B38207	WO200058469-A1	Neural/Sensory
HATCF80	B38209	WO200058469-A1	Cancer
HTOAK03	B38215	WO200058469-A1	Cancer
HSLAB11	B38216	WO200058469-A1	Cancer
HSJAU93	B38218	WO200058469-A1	Cancer
HSGBT15	B38221	WO200058469-A1	Cancer
HSLCS31	B38234	WO200058469-A1	Cancer
HSLCS34	B38235	WO200058469-A1	Cancer

HOBNF51	B38237	WO200058469-A1	Cancer
HSFAM19	B38242	WO200058469-A1	Cancer
HNHEY29	B38245	WO200058469-A1	Immune/Hematopoietic
HTHDB20	B38248	WO200058469-A1	Immune/Hematopoietic
HPMGM06	B38250	WO200058469-A1	Digestive, Neural/Sensory, Reproductive
HDPMA04	B38321	WO200061623-A1	Immune/Hematopoietic
HEMFQ46	B38322	WO200061623-A1	Cancer
HKAJK47	B38324	WO200061623-A1	Cancer
HCGMF16	B38325	WO200061623-A1	Cancer
HMSGU01	B38326	WO200061623-A1	Cancer
HNTCE26	B38327	WO200061623-A1	Cancer
HPTTI70	B38328	WO200061623-A1	Cancer
HNSAD53	B38329	WO200061623-A1	Digestive
HTEBV72	B38330	WO200061623-A1	Reproductive
HCE3Z61	B38331	WO200061623-A1	Cancer
HSSGD52	B38332	WO200061623-A1	Cancer
HAPSA79	B38333	WO200061623-A1	Cancer
HASAU84	B38334	WO200061623-A1	Cancer
HLWEA51	B38335	WO200061623-A1	Cancer
HNFI34	B38336	WO200061623-A1	Cancer
HTELS08	B38337	WO200061623-A1	Reproductive
HTLEJ24	B38338	WO200061623-A1	Cancer
HCEHF62	B38339	WO200061623-A1	Cancer
HUFBY15	B38340	WO200061623-A1	Digestive, Musculoskeletal, Reproductive
HELHD85	B38341	WO200061623-A1	Cancer
HOFNY91	B38342	WO200061623-A1	Cancer
HEGAK44	B38343	WO200061623-A1	Cancer
HETBA14	B38344	WO200061623-A1	Cancer
HBAFV19	B38345	WO200061623-A1	Cancer
HTXDO17	B38346	WO200061623-A1	Immune/Hematopoietic, Neural/Sensory, Respiratory
HE8DS15	B38347	WO200061623-A1	Cancer
HLDOW79	B38348	WO200061623-A1	Cardiovascular, Digestive
HOFND85	B38349	WO200061623-A1	Cancer
HBIBU30	B38350	WO200061623-A1	Cancer
HODFG71	B38351	WO200061623-A1	Reproductive
HNHGE28	B38352	WO200061623-A1	Cancer
HYASD09	B38355	WO200061623-A1	Cancer
HDPC63	B38356	WO200061623-A1	Cancer
HBDAD07	B38357	WO200061623-A1	Immune/Hematopoietic
HTOHG09	B38361	WO200061623-A1	Cancer
HWBFX31	B38362	WO200061623-A1	Cancer
HLHDP16	B38363	WO200061623-A1	Cancer
HSDBC88	B38364	WO200061623-A1	Cancer
HOVBX78	B38365	WO200061623-A1	Cancer
HWADJ89	B38367	WO200061623-A1	Immune/Hematopoietic
HYABE50	B38368	WO200061623-A1	Cancer
HSJAQ17	B38369	WO200061623-A1	Cancer
HCUGM86	B38370	WO200061623-A1	Immune/Hematopoietic
HLDQC46	B38371	WO200061623-A1	Cancer

HOFOA59	B38372	WO200061623-A1	Reproductive
HFABG18	B38373	WO200061623-A1	Cancer
HNHLY33	B38374	WO200061623-A1	Immune/Hematopoietic
HFCFJ18	B38375	WO200061623-A1	Cancer
HANGG89	B38376	WO200061623-A1	Cancer
HNHOD46	B38377	WO200061623-A1	Immune/Hematopoietic
HLYBI58	B38379	WO200061623-A1	Cancer
HJBG14	B38381	WO200061623-A1	Cancer
HE9NN84	B38382	WO200061623-A1	Cancer
HAPSA79	B38383	WO200061623-A1	Cancer
HAPSA79	B38384	WO200061623-A1	Cancer
HTLEJ24	B38385	WO200061623-A1	Cancer
HEGAK44	B38386	WO200061623-A1	Cancer
HTXDO17	B38387	WO200061623-A1	Immune/Hematopoietic, Neural/Sensory, Respiratory
HBIBB20	B38388	WO200061623-A1	Cancer
HSIDL71	B38389	WO200061623-A1	Cancer
HOVBX78	B38390	WO200061623-A1	Cancer
HYABE50	B38391	WO200061623-A1	Cancer
HFCFJ18	B38392	WO200061623-A1	Cancer
HPRAL78	B38394	WO200061623-A1	Cancer
HCE5F84	B38395	WO200061623-A1	Cancer
HAMHE82	B38396	WO200061623-A1	Cancer
HACBZ59	B38472	WO200061623-A1	Cancer
HACBZ59	B38475	WO200061623-A1	Cancer
HWLQU40	B38514	WO200061623-A1	Cancer
HFKFI35	B38527	WO200056882-A1	Excretory
HFPCZ55	B38529	WO200056882-A1	Cancer
HFPDR39	B38533	WO200056882-A1	Cancer
HFPDX08	B38536	WO200056882-A1	Cancer
HFRAB10	B38539	WO200056882-A1	Excretory, Immune/Hematopoietic, Neural/Sensory
HFSBE94	B38541	WO200056882-A1	Immune/Hematopoietic
HFTAR30	B38545	WO200056882-A1	Cancer
HFTBB50	B38546	WO200056882-A1	Cancer
HFSAY91	B38551	WO200056882-A1	Cancer
HFSBC10	B38552	WO200056882-A1	Immune/Hematopoietic, Mixed Fetal
HFTDK11	B38555	WO200056882-A1	Cancer
HFVHW43	B38560	WO200056882-A1	Digestive
HFXBI64	B38567	WO200056882-A1	Neural/Sensory
HFXBL05	B38568	WO200056882-A1	Mixed Fetal, Neural/Sensory
HHGBV02	B38971	WO200056880-A1	Immune/Hematopoietic, Reproductive
HHGBW55	B38972	WO200056880-A1	Immune/Hematopoietic, Reproductive
HHGDI12	B38976	WO200056880-A1	Neural/Sensory
HHPBG90	B38983	WO200056880-A1	Cancer
HHPFP26	B38987	WO200056880-A1	Cancer
HHPGU74	B38990	WO200056880-A1	Neural/Sensory
HHPEB61	B38991	WO200056880-A1	Cancer
HHSBJ92	B39002	WO200056880-A1	Cancer
HHPSE03	B39004	WO200056880-A1	Neural/Sensory



HHSCQ67	B39005	WO200056880-A1	Cancer
HHSDB43	B39007	WO200056880-A1	Cancer
HHTLH79	B39015	WO200056880-A1	Immune/Hematopoietic, Musculoskeletal, Neural/Sensory
HIABC70	B39016	WO200056880-A1	Cancer
HIBCR82	B39017	WO200056880-A1	Mixed Fetal, Neural/Sensory
HIBEC45	B39019	WO200056880-A1	Cancer
HILCA24	B39020	WO200056880-A1	Digestive, Immune/Hematopoietic, Reproductive
HLHBS54	B39093	WO200058513-A1	Cancer
HLMCT95	B39098	WO200058513-A1	Cancer
HLDRU08	B39100	WO200058513-A1	Cancer
HLDXF43	B39101	WO200058513-A1	Cancer
HLEAA10	B39102	WO200058513-A1	Immune/Hematopoietic
HLHCB33	B39104	WO200058513-A1	Digestive, Reproductive, Respiratory
HLHCF14	B39105	WO200058513-A1	Connective/Epithelial, Respiratory
HLHCN51	B39107	WO200058513-A1	Digestive, Immune/Hematopoietic, Respiratory
HLHDM38	B39109	WO200058513-A1	Cancer
HLHEX62	B39111	WO200058513-A1	Excretory, Immune/Hematopoietic, Respiratory
HLHSG15	B39114	WO200058513-A1	Cancer
HLIBD74	B39117	WO200058513-A1	Digestive
HLICO10	B39120	WO200058513-A1	Cancer
HLJBI22	B39121	WO200058513-A1	Cancer
HLLEX95	B39123	WO200058513-A1	Immune/Hematopoietic
HLMBZ14	B39127	WO200058513-A1	Immune/Hematopoietic
HLMDH01	B39129	WO200058513-A1	Immune/Hematopoietic
HLMDU23	B39130	WO200058513-A1	Immune/Hematopoietic
HLMFU53	B39133	WO200058513-A1	Cancer
HLMHG68	B39135	WO200058513-A1	Cancer
HLMIM84	B39137	WO200058513-A1	Cancer
HLMIQ83	B39139	WO200058513-A1	Immune/Hematopoietic
HLDRT09	B39140	WO200058513-A1	Cancer
HE9EA10	B39181	WO200056754-A1	Cancer
HE6CS65	B39182	WO200056754-A1	Cancer
HE8BE20	B39190	WO200056754-A1	Cancer
HE8BT58	B39193	WO200056754-A1	Cancer
HE8CA13	B39195	WO200056754-A1	Cancer
HE8FC10	B39201	WO200056754-A1	Immune/Hematopoietic, Mixed Fetal, Reproductive
HE8FG24	B39202	WO200056754-A1	Cancer
HE8FL24	B39203	WO200056754-A1	Mixed Fetal
HE8MA27	B39204	WO200056754-A1	Cancer
HE8MG56	B39205	WO200056754-A1	Mixed Fetal
HE8QU21	B39208	WO200056754-A1	Immune/Hematopoietic, Mixed Fetal

HE8UX34	B39210	WO200056754-A1	Mixed Fetal
HE9CY05	B39216	WO200056754-A1	Mixed Fetal
HE9DG54	B39217	WO200056754-A1	Cancer
HE9DZ47	B39218	WO200056754-A1	Endocrine, Immune/Hematopoietic, Mixed Fetal
HE8EX86	B39226	WO200056754-A1	Cancer
HMEIH57	B39310	WO200057903-A2	Cardiovascular, Immune/Hematopoietic
HFEB88	B39312	WO200057903-A2	Cancer
HMEKW44	B39317	WO200057903-A2	Cardiovascular, Immune/Hematopoietic, Neural/Sensory
HMELM75	B39318	WO200057903-A2	Cancer
HMIAC52	B39322	WO200057903-A2	Cancer
HMIAL39	B39325	WO200057903-A2	Cancer
HMIBD93	B39329	WO200057903-A2	Cancer
HMIBE95	B39330	WO200057903-A2	Neural/Sensory
HMMAL32	B39344	WO200057903-A2	Immune/Hematopoietic
HMMBK55	B39348	WO200057903-A2	Immune/Hematopoietic
HMMBR63	B39350	WO200057903-A2	Cancer
HMMBS55	B39351	WO200057903-A2	Immune/Hematopoietic, Reproductive
HMMBT47	B39352	WO200057903-A2	Immune/Hematopoietic
HMMCD35	B39353	WO200057903-A2	Immune/Hematopoietic
HFKCZ13	B39362	WO200057903-A2	Cancer
HFKCZ13	B39363	WO200057903-A2	Cancer
HOAAL10	B39402	WO200058340-A2	Musculoskeletal
HTXCV44	B39406	WO200058340-A2	Immune/Hematopoietic, Neural/Sensory
HTXDJ75	B39407	WO200058340-A2	Digestive, Immune/Hematopoietic, Mixed Fetal
HSIDZ25	B39410	WO200058340-A2	Cancer
HTXEN33	B39413	WO200058340-A2	Immune/Hematopoietic, Reproductive
HJPKD61	B39419	WO200058340-A2	Cancer
HNHBM16	B39420	WO200058340-A2	Immune/Hematopoietic, Neural/Sensory
HNHDE58	B39422	WO200058340-A2	Cancer
HTTDT67	B39425	WO200058340-A2	Cancer
HTLEP55	B39427	WO200058340-A2	Cancer
HCUBA28	B39430	WO200058340-A2	Cancer
HSNAT08	B39433	WO200058340-A2	Cancer
HTOEV01	B39437	WO200058340-A2	Immune/Hematopoietic, Reproductive
HSQBF66	B39439	WO200058340-A2	Cancer
HSJBY32	B39445	WO200058340-A2	Immune/Hematopoietic, Musculoskeletal, Neural/Sensory
HPMCV30	B39447	WO200058340-A2	Cancer
HPMDF45	B39448	WO200058340-A2	Excretory, Immune/Hematopoietic, Reproductive
HPIBI40	B40153	WO200058496-A1	Cancer
HRDBA20	B40154	WO200058496-A1	Musculoskeletal

HTJMA64	B40156	WO200058496-A1	Cancer
HTHDF86	B40157	WO200058496-A1	Immune/Hematopoietic
HRDBA20	B40158	WO200058496-A1	Musculoskeletal
HSAAN03	B40161	WO200058496-A1	Cancer
HRABZ80	B40162	WO200058496-A1	Excretory, Immune/Hematopoietic, Musculoskeletal
HPDDQ28	B40163	WO200058496-A1	Endocrine, Musculoskeletal
HOSBX14	B40166	WO200058496-A1	Immune/Hematopoietic, Musculoskeletal, Reproductive
HCRAI29	B40168	WO200058496-A1	Neural/Sensory
HSAXI10	B40171	WO200058496-A1	Digestive, Immune/Hematopoietic
HTSFV18	B40172	WO200058496-A1	Cancer
HNHGK22	B40174	WO200058496-A1	Immune/Hematopoietic
HPMFP48	B40176	WO200058496-A1	Cancer
HUSGL67	B40177	WO200058496-A1	Cancer
HOFMO16	B40180	WO200058496-A1	Reproductive
HPMAI31	B40182	WO200058496-A1	Cancer
HTTAP37	B40184	WO200058496-A1	Immune/Hematopoietic, Reproductive
HSABA15	B40187	WO200058496-A1	Cancer
HPEAA65	B40188	WO200058496-A1	Digestive, Immune/Hematopoietic, Reproductive
HSSAN03	B40189	WO200058496-A1	Cancer
HTTDG27	B40194	WO200058496-A1	Reproductive
HTPDV75	B40195	WO200058496-A1	Digestive, Reproductive
HMJAC12	B40197	WO200058496-A1	Neural/Sensory
HTLEJ24	B40198	WO200058496-A1	Cancer
HBAMC47	B43415	WO200055350-A1	Excretory
HBGDH11	B43571	WO200055350-A1	Cancer
HCHAK72	B43572	WO200055350-A1	Cancer
HSRAB84	B43594	WO200055350-A1	Cancer
HMWBH91	B43612	WO200055350-A1	Cancer
HUFFG07	B43619	WO200055350-A1	Cancer
HMEIY69	B43625	WO200055350-A1	Cancer
HIBCN93	B43651	WO200055350-A1	Cancer
HDPQE64	B43656	WO200055350-A1	Cancer
HSYCY88	B43723	WO200055350-A1	Cancer
HCMSD61	B43779	WO200055350-A1	Cancer
H2CBK94	B43801	WO200055350-A1	Digestive, Neural/Sensory, Respiratory
HE9NK60	B43812	WO200055350-A1	Cancer
HTXDT74	B43844	WO200055350-A1	Cancer
HTTKN45	B43848	WO200055350-A1	Cancer
HSSGC06	B43861	WO200055350-A1	Cancer
HE8AM92	B43893	WO200055350-A1	Cancer
HWLHZ28	B43933	WO200055350-A1	Cancer
HCEDM42	B43935	WO200055350-A1	Cancer
HMWFM73	B43952	WO200055350-A1	Cancer
HCHBQ27	B44028	WO200055350-A1	Reproductive

HCHBQ27	B44029	WO200055350-A1	Reproductive
HCEGS49	B44338	WO200058358-A1	Connective/Epithelial, Neural/Sensory, Reproductive
HTODH57	B44349	WO200058358-A1	Immune/Hematopoietic
HRTAR24	B44351	WO200058358-A1	Digestive, Immune/Hematopoietic
HSLAW59	B44355	WO200058358-A1	Immune/Hematopoietic, Musculoskeletal
HODAG07	B44358	WO200058358-A1	Reproductive
HODBA45	B44360	WO200058358-A1	Reproductive
HODBD79	B44361	WO200058358-A1	Immune/Hematopoietic, Reproductive
HODDG72	B44363	WO200058358-A1	Cancer
HSVAE42	B44367	WO200058358-A1	Connective/Epithelial, Neural/Sensory
HNHAG83	B44369	WO200058358-A1	Immune/Hematopoietic, Mixed Fetal, Musculoskeletal
HSIDA39	B44374	WO200058358-A1	Digestive
HYBAW56	B44375	WO200058358-A1	Musculoskeletal
HSATI91	B44376	WO200058358-A1	Immune/Hematopoietic
HPWAO89	B44379	WO200058358-A1	Immune/Hematopoietic, Reproductive
HOUCD12	B44380	WO200058358-A1	Connective/Epithelial
HWTAM38	B44381	WO200058358-A1	Digestive, Immune/Hematopoietic, Reproductive
HKGCE62	B44597	WO200058339-A2	Immune/Hematopoietic
HKGAK45	B44601	WO200058339-A2	Musculoskeletal, Reproductive
HKGBC33	B44602	WO200058339-A2	Immune/Hematopoietic
HKGBH54	B44606	WO200058339-A2	Cancer
HKGCK41	B44608	WO200058339-A2	Cancer
HKG CX05	B44610	WO200058339-A2	Cancer
HKGDA95	B44611	WO200058339-A2	Cancer
HKIME53	B44612	WO200058339-A2	Cancer
HKIXR91	B44616	WO200058339-A2	Cancer
HKMLT89	B44625	WO200058339-A2	Excretory, Immune/Hematopoietic, Reproductive
HKMMU76	B44629	WO200058339-A2	Cancer
HL3AE69	B44635	WO200058339-A2	Cancer
HLDBV54	B44638	WO200058339-A2	Cancer
HLDNF18	B44639	WO200058339-A2	Cancer
HLDOL74	B44642	WO200058339-A2	Cancer
HKIMG23	B44643	WO200058339-A2	Cancer
HLDBV18	B44645	WO200058339-A2	Cancer
HMQBU44	B44647	WO200058339-A2	Cancer
HNHDM21	B44703	WO200058494-A1	Immune/Hematopoietic
HNHDM43	B44704	WO200058494-A1	Immune/Hematopoietic
HNHDX28	B44708	WO200058494-A1	Immune/Hematopoietic
HNHEG30	B44713	WO200058494-A1	Immune/Hematopoietic
HNHEL22	B44715	WO200058494-A1	Immune/Hematopoietic
HNHFF60	B44720	WO200058494-A1	Immune/Hematopoietic
HTHDH18	B44723	WO200058494-A1	Immune/Hematopoietic

HTHDP65	B44724	WO200058494-A1	Cancer
HTHDT25	B44725	WO200058494-A1	Immune/Hematopoietic
HTGAQ29	B44727	WO200058494-A1	Immune/Hematopoietic
HTGAS70	B44728	WO200058494-A1	Cancer
HNHGD95	B44731	WO200058494-A1	Cardiovascular, Immune/Hematopoietic
HNHGR82	B44733	WO200058494-A1	Immune/Hematopoietic
HSAYL24	B44763	WO200058336-A1	Immune/Hematopoietic
HTGDS92	B44764	WO200058336-A1	Cancer
HTWDJ17	B44767	WO200058336-A1	Cancer
HNGJL07	B44776	WO200058336-A1	Immune/Hematopoietic, Neural/Sensory
HSSGS62	B44785	WO200058336-A1	Musculoskeletal, Reproductive
HOVCC73	B44786	WO200058336-A1	Immune/Hematopoietic, Reproductive
HSAYO82	B44788	WO200058336-A1	Endocrine, Immune/Hematopoietic
HMACT74	B44791	WO200058336-A1	Immune/Hematopoietic
HMIAD75	B44792	WO200058336-A1	Neural/Sensory
HUFAO92	B44795	WO200058336-A1	Digestive, Reproductive
HTWFA21	B44799	WO200058336-A1	Immune/Hematopoietic
HSDKE82	B44802	WO200058336-A1	Neural/Sensory
HMAJS26	B44803	WO200058336-A1	Cancer
HKGAP57	B44811	WO200058336-A1	Immune/Hematopoietic
HCWUW24	B44831	WO200055176-A2	Immune/Hematopoietic
HDPAE80	B44832	WO200055176-A2	Cancer
HCUFE33	B44833	WO200055176-A2	Immune/Hematopoietic
HCUFJ09	B44834	WO200055176-A2	Cancer
HCUGR26	B44840	WO200055176-A2	Immune/Hematopoietic
HCUHM71	B44844	WO200055176-A2	Immune/Hematopoietic, Musculoskeletal
HCWAK88	B44847	WO200055176-A2	Immune/Hematopoietic
HCWFK03	B44855	WO200055176-A2	Cancer
HCWHT52	B44858	WO200055176-A2	Immune/Hematopoietic
HCWKO32	B44859	WO200055176-A2	Immune/Hematopoietic
HCWUF93	B44861	WO200055176-A2	Cancer
HDACJ52	B44865	WO200055176-A2	Cancer
HDFAB86	B44866	WO200055176-A2	Mixed Fetal, Neural/Sensory
HDHAA55	B44870	WO200055176-A2	Immune/Hematopoietic, Neural/Sensory
HDHEB12	B44871	WO200055176-A2	Immune/Hematopoietic, Neural/Sensory
HDHIA16	B44874	WO200055176-A2	Cancer
HDHIA26	B44875	WO200055176-A2	Neural/Sensory
HARNB17	B44909	WO200055176-A2	Cancer
HBKEE60	B44917	WO200055200-A1	Digestive
HBMWJ92	B44920	WO200055200-A1	Cancer
HBMXW83	B44924	WO200055200-A1	Cancer
HCE3R01	B44926	WO200055200-A1	Cancer
HBNBJ76	B44930	WO200055200-A1	Cancer
HBQAC72	B44935	WO200055200-A1	Neural/Sensory
HBSAJ63	B44937	WO200055200-A1	Cancer
HATDE03	B44938	WO200055200-A1	Cancer

HBSDD24	B44939	WO200055200-A1	Cancer
HBWBD25	B44940	WO200055200-A1	Immune/Hematopoietic, Neural/Sensory
HBXBM24	B44943	WO200055200-A1	Neural/Sensory
HBXBM78	B44944	WO200055200-A1	Cancer
HBNA40	B44947	WO200055200-A1	Cancer
HBXCQ03	B44949	WO200055200-A1	Cancer
HBXFI33	B44955	WO200055200-A1	Immune/Hematopoietic, Neural/Sensory
HBXGE12	B44958	WO200055200-A1	Cancer
HCDAA24	B44960	WO200055200-A1	Cancer
HCDAH02	B44962	WO200055200-A1	Immune/Hematopoietic, Musculoskeletal
HCDAR40	B44964	WO200055200-A1	Cardiovascular, Immune/Hematopoietic, Musculoskeletal
HCDBE76	B44965	WO200055200-A1	Cancer
HCDBO32	B44966	WO200055200-A1	Cancer
HTDAF68	B45025	WO200058357-A1	Immune/Hematopoietic
HTWFA88	B45027	WO200058357-A1	Digestive, Immune/Hematopoietic
HSDJV40	B45028	WO200058357-A1	Immune/Hematopoietic, Neural/Sensory
HSDKA64	B45029	WO200058357-A1	Immune/Hematopoietic, Neural/Sensory
HRAAC36	B45030	WO200058357-A1	Excretory, Immune/Hematopoietic
HPRCF50	B45031	WO200058357-A1	Cancer
HUSXP50	B45034	WO200058357-A1	Cardiovascular, Reproductive
HSSFP88	B45035	WO200058357-A1	Cancer
HOVBS68	B45037	WO200058357-A1	Cancer
HOVCO53	B45039	WO200058357-A1	Reproductive
HOVBI16	B45040	WO200058357-A1	Cancer
HOSFR35	B45041	WO200058357-A1	Cancer
HNTRB25	B45042	WO200058357-A1	Cancer
HUFBP77	B45043	WO200058357-A1	Cancer
HUFAP33	B45044	WO200058357-A1	Cancer
HSDHD05	B45045	WO200058357-A1	Neural/Sensory
HTWFM85	B45046	WO200058357-A1	Cancer
HSDJC96	B45048	WO200058357-A1	Cancer
HTXKH40	B45050	WO200058357-A1	Cancer
HPQCI62	B45051	WO200058357-A1	Cancer
HOFMJ65	B45053	WO200058357-A1	Cancer
HMUAN45	B45057	WO200058357-A1	Cancer
HAJAB88	B45059	WO200058357-A1	Cancer
HPTTT62	B45067	WO200058357-A1	Cancer
HSRDW57	B45071	WO200058357-A1	Cancer
HSRDW57	B45118	WO200058357-A1	Cancer
HJBCG74	B45121	WO200058467-A1	Cancer
HCECO77	B45124	WO200058467-A1	Cancer
HJABC58	B45129	WO200058467-A1	Cancer
HJMBK59	B45140	WO200058467-A1	Cancer
HJMBP01	B45141	WO200058467-A1	Cancer
HJMBW62	B45143	WO200058467-A1	Reproductive
HJPAQ19	B45146	WO200058467-A1	Cancer

HJPBN96	B45148	WO200058467-A1	Cancer
HJPBK28	B45149	WO200058467-A1	Cancer
HKABN63	B45152	WO200058467-A1	Cancer
HKAFF50	B45159	WO200058467-A1	Cancer
HKMSB01	B45166	WO200058467-A1	Cancer
HISEJ52	B45171	WO200058467-A1	Cancer
HJBCG74	B45174	WO200058467-A1	Cancer
HJBCG74	B45175	WO200058467-A1	Cancer
HOFNY15	B45227	WO200063230-A2	Reproductive
HNTAF42	B45228	WO200063230-A2	Cancer
HPJAW78	B45233	WO200063230-A2	Immune/Hematopoietic, Musculoskeletal, Reproductive
HPJBS16	B45234	WO200063230-A2	Connective/Epithelial, Reproductive
HPJCV35	B45236	WO200063230-A2	Reproductive
HSNAH56	B45239	WO200063230-A2	Cancer
HE2FE89	B45246	WO200063230-A2	Cardiovascular, Digestive, Mixed Fetal
HPVAF86	B45249	WO200063230-A2	Reproductive
HOGCD78	B45257	WO200063230-A2	Reproductive
HRABU56	B45264	WO200063230-A2	Cardiovascular, Excretory, Musculoskeletal
HCUBY47	B45267	WO200063230-A2	Digestive, Immune/Hematopoietic
HUDBE20	B45270	WO200063230-A2	Reproductive
HUDBK47	B45271	WO200063230-A2	Immune/Hematopoietic, Reproductive
HSOAH16	B45318	WO200061628-A1	Digestive
HWTBX66	B45320	WO200061628-A1	Cancer
HTXDO17	B45321	WO200061628-A1	Immune/Hematopoietic, Neural/Sensory, Respiratory
HSSDQ20	B45325	WO200061628-A1	Musculoskeletal, Neural/Sensory
HTOHM82	B45329	WO200061628-A1	Cancer
HTOIH51	B45333	WO200061628-A1	Immune/Hematopoietic
HHLBA86	B45334	WO200061628-A1	Digestive
HTAEH58	B45335	WO200061628-A1	Immune/Hematopoietic
HLTCO22	B45338	WO200061628-A1	Cancer
HTOJS23	B45343	WO200061628-A1	Immune/Hematopoietic
HNHBE21	B45356	WO200061628-A1	Immune/Hematopoietic
HSSFE38	B45387	WO200061627-A1	Cancer
HTOGB79	B45388	WO200061627-A1	Cancer
HKABU43	B45389	WO200061627-A1	Cancer
HSYBV44	B45398	WO200061627-A1	Immune/Hematopoietic
HOHBZ10	B45399	WO200061627-A1	Cancer
HWAAQ28	B45400	WO200061627-A1	Cancer
HWBBQ70	B45402	WO200061627-A1	Immune/Hematopoietic, Neural/Sensory
HWBCN36	B45403	WO200061627-A1	Immune/Hematopoietic
HWBCP16	B45404	WO200061627-A1	Immune/Hematopoietic
HWHGW09	B45406	WO200061627-A1	Cancer
HWHHA21	B45407	WO200061627-A1	Connective/Epithelial

HYABE50	B45408	WO200061627-A1	Cancer
HBXFA04	B45411	WO200061627-A1	Neural/Sensory
HPRCA64	B45412	WO200061627-A1	Cancer
HTXAA20	B45414	WO200061627-A1	Cancer
HOFAA78	B45423	WO200061627-A1	Reproductive
HTOGR38	B45427	WO200061627-A1	Immune/Hematopoietic
HUKBT67	B45431	WO200061627-A1	Cancer
HCEMU42	B45432	WO200061627-A1	Cancer
HWHPB78	B45433	WO200061627-A1	Cancer
HSYBV44	B45452	WO200061627-A1	Immune/Hematopoietic
HNHEN70	B45699	WO200071584-A1	Cancer
HLYBN81	B45700	WO200071584-A1	Cancer
H7TME50	B45701	WO200071584-A1	Cancer
HDPWP65	B45702	WO200071584-A1	Cancer
HDTIE58	B45703	WO200071584-A1	Cardiovascular, Connective/Epithelial, Immune/Hematopoietic
H7TPC96	B45704	WO200071584-A1	Cancer
H7MAD52	B45705	WO200071584-A1	Reproductive
HYASC03	B49502	WO200070076-A1	Endocrine, Immune/Hematopoietic
HCE1K90	B49503	WO200070076-A1	Cancer
HNTMH2C	B49504	WO200070076-A1	Cancer
HE8EJ16	B49505	WO200070076-A1	Mixed Fetal, Neural/Sensory, Reproductive
HFEBD57	B49506	WO200070076-A1	Cancer
HOFAD65	B49507	WO200070076-A1	Cancer
HAPRB43	B49508	WO200070076-A1	Cancer
HCE1K90	B49509	WO200070076-A1	Cancer
HE8EJ16	B49510	WO200070076-A1	Mixed Fetal, Neural/Sensory, Reproductive
HFEBD57	B49511	WO200070076-A1	Cancer
HFEBD57	B49512	WO200070076-A1	Cancer
HOFAD65	B49513	WO200070076-A1	Cancer
HFPEY75	B49533	WO200061774-A2	Cancer
HOHEC84	B49534	WO200061774-A2	Immune/Hematopoietic, Musculoskeletal
HFKCD20	B49535	WO200061774-A2	Cancer
HKMLR17	B49536	WO200061774-A2	Cancer
HTHCW70	B49537	WO200061774-A2	Cancer
HOHEC84	B49538	WO200061774-A2	Immune/Hematopoietic, Musculoskeletal
HOUCQ17	B50011	WO200071577-A1	Cancer
HMADD44	B50378	WO200061614-A2	Cancer
HDQER52	B50379	WO200061614-A2	Cancer
HTELM46	B50380	WO200061614-A2	Digestive, Immune/Hematopoietic, Reproductive
HDPUS73	B50381	WO200061614-A2	Cancer
HFCDT50	B50382	WO200061614-A2	Cancer
HEMGR64	B50383	WO200061614-A2	Cancer
HHFDM26	B50384	WO200061614-A2	Cancer
HTTIA36	B50385	WO200061614-A2	Cancer
HDQHP22	B50387	WO200061614-A2	Cancer



HRDCD90	B50388	WO200061614-A2	Cancer
HEOMG91	B50389	WO200061614-A2	Cancer
HSLGK66	B50390	WO200061614-A2	Cancer
HSIFX64	B50391	WO200061614-A2	Cancer
HETCD80	B50392	WO200061614-A2	Reproductive
HHSGB09	B50393	WO200061614-A2	Cancer
HLWBT44	B50394	WO200061614-A2	Cancer
HTLJG95	B50395	WO200061614-A2	Cancer
HDPDH32	B50935	WO200073323-A2	Immune/Hematopoietic
HHFMQ22	B50936	WO200073323-A2	Cancer
HETCM67	B50937	WO200073323-A2	Cancer
HWBDU78	B50938	WO200073323-A2	Cancer
HTXEM16	B50939	WO200073323-A2	Cancer
HBJEM23	B50940	WO200073323-A2	Cardiovascular, Musculoskeletal, Reproductive
H7TMD22	B50941	WO200073323-A2	Neural/Sensory
HDPDH32	B50942	WO200073323-A2	Immune/Hematopoietic
HDPDH32	B50943	WO200073323-A2	Immune/Hematopoietic
HSQAX94	B51382	WO200058495-A1	Cancer
HTOFA11	B51383	WO200058495-A1	Cancer
HFFAH01	B51384	WO200058495-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HNHBI65	B51385	WO200058495-A1	Immune/Hematopoietic
HNHCP14	B51386	WO200058495-A1	Immune/Hematopoietic
HEAAW54	B51387	WO200058495-A1	Reproductive
HSRDM56	B51393	WO200058495-A1	Cancer
HSAXL82	B51397	WO200058495-A1	Immune/Hematopoietic
HCE3L04	B51398	WO200058495-A1	Neural/Sensory
HEBGM06	B51402	WO200058495-A1	Cancer
HTWBO30	B51403	WO200058495-A1	Cancer
HSSJF26	B51404	WO200058495-A1	Musculoskeletal
HUKAD46	B51412	WO200058495-A1	Endocrine, Immune/Hematopoietic, Reproductive
HPDDT14	B51413	WO200058495-A1	Cancer
HTEDF78	B51415	WO200058495-A1	Reproductive
HSUSB73	B51416	WO200058495-A1	Immune/Hematopoietic, Reproductive
HSRAA81	B51417	WO200058495-A1	Cancer
HCABW10	B51420	WO200058495-A1	Cancer
HTWAM19	B51422	WO200058495-A1	Immune/Hematopoietic
HSDZO08	B51620	WO200061620-A1	Cancer
HSLHX15	B51624	WO200061620-A1	Musculoskeletal
HSNBM34	B51625	WO200061620-A1	Digestive
HSWBE76	B51629	WO200061620-A1	Cancer
HSXAS59	B51630	WO200061620-A1	Neural/Sensory
HSXAY60	B51631	WO200061620-A1	Cancer
HTEDX07	B51635	WO200061620-A1	Cancer
HTEJY20	B51640	WO200061620-A1	Cancer
HLDQU79	B51645	WO200061620-A1	Cancer
HTLBT80	B51646	WO200061620-A1	Cancer
HTEAF65	B51648	WO200061620-A1	Excretory, Reproductive
HTNBJ15	B51649	WO200061620-A1	Cancer

HOUPE77	B51650	WO200061620-A1	Cancer
HTOJL95	B51651	WO200061620-A1	Cancer
HTTDN24	B51653	WO200061620-A1	Cancer
HTXAD75	B51655	WO200061620-A1	Cancer
HTXDJ21	B51657	WO200061620-A1	Immune/Hematopoietic
HKB1E57	B51658	WO200061620-A1	Cancer
HAQBZ15	B51659	WO200061620-A1	Cancer
HMWAB92	B51661	WO200061620-A1	Cancer
HSNBL85	B51663	WO200061620-A1	Cancer
HWBDJ08	B51664	WO200061620-A1	Cancer
HYABC84	B51667	WO200061620-A1	Cancer
HTEDX07	B51682	WO200061620-A1	Cancer
HOUHQ36	B51724	WO200061625-A1	Connective/Epithelial
HOUIG92	B51726	WO200061625-A1	Cancer
HSAZP90	B51728	WO200061625-A1	Immune/Hematopoietic
HSPAY90	B51730	WO200061625-A1	Cancer
HWHPU44	B51731	WO200061625-A1	Connective/Epithelial
HWACZ33	B51734	WO200061625-A1	Digestive, Immune/Hematopoietic, Reproductive
HRADA42	B51736	WO200061625-A1	Cancer
HRADN25	B51737	WO200061625-A1	Cancer
HRADN25	B51738	WO200061625-A1	Cancer
HRADT25	B51739	WO200061625-A1	Digestive, Excretory
HRADT25	B51740	WO200061625-A1	Digestive, Excretory
HOHEC84	B51741	WO200061625-A1	Immune/Hematopoietic, Musculoskeletal
HRADU15	B51742	WO200061625-A1	Excretory
HWDAG96	B51743	WO200061625-A1	Cancer
HWD AJ01	B51745	WO200061625-A1	Connective/Epithelial
HMBSF85	B51749	WO200061625-A1	Cancer
HRGSE38	B51752	WO200061625-A1	Cancer
HTLBF46	B51755	WO200061625-A1	Cancer
HSRHB59	B51761	WO200061625-A1	Cancer
HRDDQ39	B51762	WO200061625-A1	Cancer
HJPCH08	B51766	WO200061625-A1	Cancer
HOEBJ70	B51767	WO200061625-A1	Cancer
HDQER52	B51795	WO200061625-A1	Cancer
HTLBF63	B51827	WO200061626-A1	Cancer
HTOAT56	B51828	WO200061626-A1	Cancer
HSSMY35	B51829	WO200061626-A1	Cancer
HBHAA81	B51840	WO200061626-A1	Cancer
HOQBG21	B51841	WO200061626-A1	Cancer
HTPCG10	B51842	WO200061626-A1	Cancer
HSAX04	B51848	WO200061626-A1	Cancer
HPEAD23	B51850	WO200061626-A1	Cancer
HODDN21	B51857	WO200061626-A1	Reproductive
HOABH36	B51859	WO200061626-A1	Cancer
HOACG07	B51860	WO200061626-A1	Cancer
HPMDA80	B51864	WO200061626-A1	Cancer
HSKCQ51	B51865	WO200061626-A1	Cancer
HRDDS22	B51870	WO200061626-A1	Cancer
HTXDT72	B51871	WO200061626-A1	Cancer

HTXDG92	B51873	WO200061626-A1	Cancer
HTXES13	B51874	WO200061626-A1	Cancer
HSSEF77	B51875	WO200061626-A1	Cancer
HTEGH03	B51883	WO200061626-A1	Cancer
HLMJB64	B51933	WO200058334-A1	Cancer
HLMNA19	B51936	WO200058334-A1	Cardiovascular, Immune/Hematopoietic
HLQAM30	B51937	WO200058334-A1	Cancer
HLQCX36	B51942	WO200058334-A1	Digestive
HLQCY09	B51943	WO200058334-A1	Digestive
HLQDM47	B51947	WO200058334-A1	Digestive
HLQDU77	B51948	WO200058334-A1	Cancer
HLTDA14	B51952	WO200058334-A1	Immune/Hematopoietic
HLTDK30	B51955	WO200058334-A1	Cancer
HLTDX04	B51958	WO200058334-A1	Cancer
HLWU42	B51964	WO200058334-A1	Cancer
HLWAW73	B51965	WO200058334-A1	Cancer
HLWAX50	B51966	WO200058334-A1	Cancer
HLWBJ93	B51968	WO200058334-A1	Cancer
HLWCN37	B51970	WO200058334-A1	Cancer
HLYAL28	B51975	WO200058334-A1	Immune/Hematopoietic
HFXDR47	B52012	WO200061596-A1	Cancer
HNHBB10	B52017	WO200061596-A1	Immune/Hematopoietic, Reproductive
HPTRI42	B52019	WO200061596-A1	Cancer
HTWCE14	B52020	WO200061596-A1	Cancer
HPTVH59	B52025	WO200061596-A1	Endocrine, Neural/Sensory
HUSGU40	B52027	WO200061596-A1	Cancer
HUSYG26	B52028	WO200061596-A1	Cancer
HOVCJ71	B52029	WO200061596-A1	Reproductive
HSKYR49	B52034	WO200061596-A1	Cancer
HTWEG06	B52040	WO200061596-A1	Immune/Hematopoietic
HSDJF04	B52041	WO200061596-A1	Cancer
HPQAN50	B52044	WO200061596-A1	Reproductive
HT5FX76	B52051	WO200061596-A1	Cancer
HT5FX79	B52052	WO200061596-A1	Cancer
HNTRQ40	B52053	WO200061596-A1	Cancer
HOUFS04	B52057	WO200061596-A1	Cancer
HOGAR71	B52059	WO200061596-A1	Cancer
HOFNB74	B52060	WO200061596-A1	Reproductive
HOGAR71	B52101	WO200061596-A1	Cancer
H7TDB54	B52104	WO200061624-A1	Cancer
HOSEM81	B52105	WO200061624-A1	Cancer
HTXKF95	B52108	WO200061624-A1	Cancer
HTGGM44	B52113	WO200061624-A1	Immune/Hematopoietic, Musculoskeletal
HROBJ10	B52114	WO200061624-A1	Cancer
HTXLC05	B52118	WO200061624-A1	Digestive, Immune/Hematopoietic, Respiratory
HTXLC45	B52119	WO200061624-A1	Immune/Hematopoietic
HNHLD80	B52120	WO200061624-A1	Immune/Hematopoietic
HNGKT41	B52124	WO200061624-A1	Immune/Hematopoietic
HNHMP15	B52125	WO200061624-A1	Immune/Hematopoietic
HNHMY76	B52127	WO200061624-A1	Immune/Hematopoietic,

			Reproductive
HNHND14	B52129	WO200061624-A1	Immune/Hematopoietic
HNHOF09	B52131	WO200061624-A1	Immune/Hematopoietic
HODEM38	B52132	WO200061624-A1	Digestive, Immune/Hematopoietic, Reproductive
HNGMW45	B52137	WO200061624-A1	Immune/Hematopoietic
HNGNK44	B52139	WO200061624-A1	Immune/Hematopoietic
HTLGL33	B52145	WO200061624-A1	Reproductive
HTLGY50	B52146	WO200061624-A1	Cancer
HNGKY94	B52147	WO200061624-A1	Immune/Hematopoietic
HTXNV66	B52150	WO200061624-A1	Cancer
HRODG74	B53274	WO200055351-A1	Cancer
HTTDO45	B53323	WO200055351-A1	Cancer
HSIFY77	B53335	WO200055351-A1	Cancer
HWMIW26	B53358	WO200055351-A1	Cancer
HEAHA84	B53397	WO200055351-A1	Cancer
HBKDN33	B53414	WO200055351-A1	Cancer
HKAIL83	B53430	WO200055351-A1	Cancer
HBMSK08	B53503	WO200055351-A1	Cancer
HTELE03	B53617	WO200055351-A1	Cancer
HSWAR63	B53774	WO200055351-A1	Reproductive
HFXAM85	B54142	WO200055320-A1	Cancer
HISCO10	B54185	WO200055320-A1	Digestive
HISBT02	B54226	WO200055320-A1	Digestive
HNHLV34	B54251	WO200055320-A1	Cancer
HUSXO71	B54257	WO200055320-A1	Cardiovascular, Immune/Hematopoietic, Reproductive
HLWBY67	B54277	WO200055320-A1	Cancer
HUVDP63	B54282	WO200055320-A1	Cancer
HSTAH26	B54290	WO200055320-A1	Connective/Epithelial
HWLXE16	B54305	WO200055320-A1	Digestive
HDQEG93	B54316	WO200055320-A1	Cancer
HSLJG12	B54341	WO200055320-A1	Cancer
HAOSL81	B54358	WO200055320-A1	Cancer
HOFNH33	B54374	WO200055320-A1	Reproductive
HAJBV26	B56077	WO200070042-A1	Cancer
HAPOC74	B56078	WO200070042-A1	Excretory, Immune/Hematopoietic, Reproductive
HATEI47	B56079	WO200070042-A1	Endocrine
HNHGD15	B56080	WO200070042-A1	Immune/Hematopoietic
HRKAB52	B56081	WO200070042-A1	Cancer
HKGAT94	B56082	WO200070042-A1	Digestive, Reproductive
HODAH46	B56083	WO200070042-A1	Cancer
HASCE69	B56084	WO200070042-A1	Cancer
HBNBE21	B56085	WO200070042-A1	Cancer
HFLSH80	B56086	WO200070042-A1	Cancer
HRACM44	B56087	WO200070042-A1	Excretory, Immune/Hematopoietic
HBXFR04	B56090	WO200070042-A1	Neural/Sensory
HNHFM14	B56094	WO200070042-A1	Cancer
HEBCM27	B56095	WO200070042-A1	Cancer
HNHBM80	B56096	WO200070042-A1	Immune/Hematopoietic

			Reproductive
HTEAR66	B56098	WO200070042-A1	Reproductive
HTLDW38	B56099	WO200070042-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HTOFD28	B56100	WO200070042-A1	Immune/Hematopoietic
HFPBW41	B56101	WO200070042-A1	Neural/Sensory
HTSAC80	B56102	WO200070042-A1	Cancer
HANGD45	B56103	WO200070042-A1	Musculoskeletal
HNGJL11	B56106	WO200070042-A1	Immune/Hematopoietic, Musculoskeletal
HYBBE75	B56108	WO200070042-A1	Musculoskeletal
HCWDS72	B56109	WO200070042-A1	Cancer
HKPAD17	B56110	WO200070042-A1	Excretory
HTLGY87	B56114	WO200070042-A1	Cancer
HULAG01	B56115	WO200070042-A1	Cardiovascular
HYAAJ71	B56116	WO200070042-A1	Immune/Hematopoietic
HE9HY07	B56118	WO200070042-A1	Mixed Fetal, Reproductive
HLSAF81	B56121	WO200070042-A1	Cancer
HNGAU09	B56123	WO200070042-A1	Immune/Hematopoietic
HTEID16	B56124	WO200070042-A1	Reproductive
HTWKE60	B56125	WO200070042-A1	Immune/Hematopoietic
HHLAB61	B56127	WO200070042-A1	Digestive
HRLMB56	B56128	WO200070042-A1	Cancer
HCUDW10	B56131	WO200070042-A1	Cancer
HNGIQ46	B56133	WO200070042-A1	Immune/Hematopoietic
H7TMD22	B56135	WO200070042-A1	Neural/Sensory
HHFCJ31	B56136	WO200070042-A1	Cardiovascular, Connective/Epithelial, Reproductive
HLDBW08	B56137	WO200070042-A1	Digestive
H6EEW15	B56139	WO200070042-A1	Cancer
HNHBM26	B56142	WO200070042-A1	Immune/Hematopoietic, Reproductive
HFIVA74	B56144	WO200070042-A1	Musculoskeletal, Reproductive
HPWAG46	B56145	WO200070042-A1	Cancer
HBJFM34	B56146	WO200070042-A1	Immune/Hematopoietic
HRDDS01	B56147	WO200070042-A1	Musculoskeletal
HGBDH53	B56152	WO200070042-A1	Cancer
HMKCV28	B56154	WO200070042-A1	Neural/Sensory
HPLAT69	B56155	WO200070042-A1	Cancer
HNGBJ27	B56157	WO200070042-A1	Immune/Hematopoietic
HFXHO83	B56158	WO200070042-A1	Cancer
HKGAM07	B56159	WO200070042-A1	Digestive, Endocrine
HTXFE73	B56160	WO200070042-A1	Cancer
HEBBN36	B56163	WO200070042-A1	Cancer
HJPCY06	B56164	WO200070042-A1	Cancer
HTEGS19	B56165	WO200070042-A1	Cancer
HMJAK63	B56166	WO200070042-A1	Neural/Sensory
HNHGE75	B56170	WO200070042-A1	Immune/Hematopoietic
HMELA16	B56171	WO200070042-A1	Cardiovascular, Immune/Hematopoietic
HNGAJ15	B56172	WO200070042-A1	Immune/Hematopoietic,

			Neural/Sensory
HNHHD40	B56173	WO200070042-A1	Cancer
HFPAA06	B56177	WO200070042-A1	Cancer
HNGIH43	B56180	WO200070042-A1	Immune/Hematopoietic, Reproductive
HLSAD65	B56182	WO200070042-A1	Cancer
HMDAK33	B56183	WO200070042-A1	Neural/Sensory
HNALC70	B56184	WO200070042-A1	Cancer
HOUCW42	B56185	WO200070042-A1	Connective/Epithelial
HLMMX46	B56186	WO200070042-A1	Immune/Hematopoietic
HHSDI68	B56188	WO200070042-A1	Neural/Sensory
HLMIV11	B56190	WO200070042-A1	Immune/Hematopoietic
HBMCI50	B56193	WO200070042-A1	Immune/Hematopoietic
HCRAI47	B56195	WO200070042-A1	Cancer
HNHEU34	B56198	WO200070042-A1	Immune/Hematopoietic
HPFCR15	B56199	WO200070042-A1	Digestive, Mixed Fetal, Reproductive
HNGJM27	B56201	WO200070042-A1	Immune/Hematopoietic
HNHEL19	B56202	WO200070042-A1	Immune/Hematopoietic, Reproductive
HGOC38	B56204	WO200070042-A1	Cancer
HHGCG53	B56205	WO200070042-A1	Cancer
HHSBJ93	B56206	WO200070042-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HLHDS79	B56208	WO200070042-A1	Cancer
HNHEA64	B56209	WO200070042-A1	Immune/Hematopoietic
HOVAZ13	B56211	WO200070042-A1	Cancer
HHGBK24	B56214	WO200070042-A1	Cancer
HILCG67	B56215	WO200070042-A1	Cancer
HOSFC36	B56218	WO200070042-A1	Cancer
HKGAT94	B56220	WO200070042-A1	Digestive, Reproductive
HBNBE21	B56221	WO200070042-A1	Cancer
HFLSH80	B56222	WO200070042-A1	Cancer
HGBDH53	B56227	WO200070042-A1	Cancer
HMELA16	B56230	WO200070042-A1	Cardiovascular, Immune/Hematopoietic
HNGAJ15	B56231	WO200070042-A1	Immune/Hematopoietic, Neural/Sensory
HCRAI47	B56232	WO200070042-A1	Cancer
HNHEL19	B56233	WO200070042-A1	Immune/Hematopoietic, Reproductive
HOSFC36	B56236	WO200070042-A1	Cancer
HHSBJ93	B56351	WO200070042-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HJAAT54	B56364	WO200055174-A1	Cancer
HPCQS73	B56431	WO200055174-A1	Cancer
HPRTL26	B56435	WO200055174-A1	Reproductive
HPFEA08	B56518	WO200055174-A1	Reproductive
HTEPF49	B56536	WO200055174-A1	Cancer
HOFAC67	B56601	WO200055174-A1	Reproductive
HNTBH70	B56606	WO200055174-A1	Cancer
HMQAI69	B56630	WO200055174-A1	Cancer

HDPBI36	B56671	WO200055174-A1	Cancer
HL2AH06	B56687	WO200055174-A1	Immune/Hematopoietic
HHEPI21	B56725	WO200055174-A1	Cancer
HAIBC14	B56739	WO200055174-A1	Cancer
HDQGM08	B56749	WO200055174-A1	Cancer
HWHPD31	B56788	WO200055174-A1	Cancer
HPJCY94	B56791	WO200055174-A1	Musculoskeletal, Reproductive
HDSAK19	B56816	WO200055174-A1	Cancer
HTTFG83	B56820	WO200055174-A1	Reproductive
HDPVR90	B56824	WO200055174-A1	Cancer
HSLJW05	B56860	WO200055174-A1	Cancer
HHEPE84	B56876	WO200055174-A1	Cancer
HE2LW65	B56909	WO200055174-A1	Cancer
HPRTI79	B56925	WO200055174-A1	Cancer
HEQAN39	B56926	WO200055174-A1	Cancer
HEMFC70	B56931	WO200055174-A1	Cancer
HELGM94	B56937	WO200055174-A1	Cancer
HDHMB78	B56950	WO200055174-A1	Cancer
HDPQE64	B56987	WO200055174-A1	Cancer
HDPBQ32	B56996	WO200055174-A1	Cancer
HBXGB85	B57056	WO200055174-A1	Neural/Sensory
HBUAC02	B57061	WO200055174-A1	Cancer
HOCOT88	B57077	WO200055174-A1	Cancer
HAJAT72	B57101	WO200055174-A1	Reproductive
HPJAV43	B57106	WO200055174-A1	Immune/Hematopoietic, Reproductive, Respiratory
HRODJ79	B57121	WO200055174-A1	Cancer
HE8FD92	B57128	WO200055174-A1	Cancer
HETAM53	B57137	WO200055174-A1	Cancer
HCHBQ27	B57159	WO200055174-A1	Reproductive
HAPSG03	B58194	WO200055180-A2	Cancer
HHFFR04	B58240	WO200055180-A2	Cancer
HCLSC85	B58320	WO200055180-A2	Respiratory
HPAMC60	B58368	WO200055180-A2	Cancer
HCE5E24	B58386	WO200055180-A2	Cancer
HMADJ17	B58396	WO200055180-A2	Cancer
HADCL25	B58403	WO200055180-A2	Cancer
HWD AO40	B58434	WO200055180-A2	Cancer
HRGBG45	B58718	WO200055173-A1	Cancer
HOFMM27	B58772	WO200055173-A1	Reproductive
HOFMH95	B58829	WO200055173-A1	Reproductive
HEMFK40	B58912	WO200055173-A1	Cancer
HODBC01	B58913	WO200055173-A1	Reproductive
HOGAV29	B58914	WO200055173-A1	Immune/Hematopoietic, Reproductive
HOFNL25	B58917	WO200055173-A1	Reproductive
HOFMH12	B58929	WO200055173-A1	Reproductive
HOFOC33	B58932	WO200055173-A1	Reproductive
HSIFL06	B58996	WO200055173-A1	Cancer
HNHBE38	B59469	WO200077173-A1	Cancer
HOPBP13	B59470	WO200077173-A1	Cancer
HOUDE92	B59472	WO200077173-A1	Cancer
HPQAJ27	B59473	WO200077173-A1	Cancer

HTLEV48	B59476	WO200077173-A1	Reproductive
HSPAI20	B59478	WO200077173-A1	Digestive, Neural/Sensory
HSPAA89	B59479	WO200077173-A1	Digestive
HTWEH94	B59482	WO200077173-A1	Immune/Hematopoietic
HTEGS48	B59490	WO200077173-A1	Reproductive
HTEIV65	B59491	WO200077173-A1	Reproductive
HOSEI81	B59494	WO200077173-A1	Digestive, Musculoskeletal
HOEFL74	B59495	WO200077173-A1	Cardiovascular, Digestive, Musculoskeletal
HOGAA41	B59497	WO200077173-A1	Cancer
HOFMT59	B59499	WO200077173-A1	Reproductive
HSYBD33	B59502	WO200077173-A1	Immune/Hematopoietic
HTOHO21	B59503	WO200077173-A1	Immune/Hematopoietic
HTAED89	B59504	WO200077173-A1	Immune/Hematopoietic
HUSCA09	B60703	WO200076531-A1	Cancer
HBXCD59	B60705	WO200076531-A1	Immune/Hematopoietic, Neural/Sensory
HDPSZ07	B60706	WO200076531-A1	Immune/Hematopoietic
HCLCU75	B60712	WO200076531-A1	Respiratory
HDABR74	B60714	WO200076531-A1	Cancer
HSSEA64	B60721	WO200076531-A1	Cancer
HSSJF96	B60722	WO200076531-A1	Musculoskeletal
HT4FV41	B60724	WO200076531-A1	Cancer
HBSAJ63	B60725	WO200076531-A1	Cancer
HTODA92	B60726	WO200076531-A1	Cancer
HTTCB60	B60727	WO200076531-A1	Cancer
HTTEO25	B60728	WO200076531-A1	Cancer
HTXDD61	B60733	WO200076531-A1	Cancer
HTXJM94	B60734	WO200076531-A1	Cancer
HWHRC51	B60736	WO200076531-A1	Cancer
HAGFJ67	B60737	WO200076531-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HBSAK32	B60739	WO200076531-A1	Cancer
HCHCG33	B60741	WO200076531-A1	Cancer
HE8FD92	B60747	WO200076531-A1	Cancer
HSAWB58	B63049	WO200061748-A1	Immune/Hematopoietic
HCE1T53	B63050	WO200061748-A1	Neural/Sensory
HODCY44	B63057	WO200061748-A1	Reproductive
HTEJF31	B63072	WO200061748-A1	Reproductive
HTPCW21	B63077	WO200061748-A1	Digestive, Neural/Sensory
HPFBA54	B63082	WO200061748-A1	Reproductive
HSABG81	B63083	WO200061748-A1	Cancer
HTECB02	B63084	WO200061748-A1	Cancer
HTEDJ28	B63086	WO200061748-A1	Cancer
HSLEC18	B63092	WO200061748-A1	Cancer
HTSGO13	B63095	WO200061748-A1	Cancer
HTLEM16	B63096	WO200061748-A1	Cancer
HTXPD86	B63136	WO200061629-A1	Cancer
HWLGP26	B63137	WO200061629-A1	Cancer
HAJAY92	B63141	WO200061629-A1	Cancer
HSYBZ94	B63145	WO200061629-A1	Cancer



HPRBH85	B63146	WO200061629-A1	Cancer
HTECE87	B63147	WO200061629-A1	Cancer
HNHGD95	B63148	WO200061629-A1	Cardiovascular, Immune/Hematopoietic
HNKAA76	B63150	WO200061629-A1	Cancer
HOABP31	B63152	WO200061629-A1	Cancer
HOHBC57	B63154	WO200061629-A1	Cancer
HOSCZ41	B63156	WO200061629-A1	Cancer
HMVEC89	B63157	WO200061629-A1	Cancer
HPJB51	B63162	WO200061629-A1	Cancer
HGBBR29	B63164	WO200061629-A1	Cancer
HPMDD27	B63165	WO200061629-A1	Cancer
HPRCM72	B63169	WO200061629-A1	Cancer
HPTRM02	B63171	WO200061629-A1	Cancer
HPTRW28	B63172	WO200061629-A1	Cancer
HRAAZ12	B63175	WO200061629-A1	Cancer
HRDEX93	B63177	WO200061629-A1	Cancer
HRDFE30	B63178	WO200061629-A1	Cancer
HBJHT01	B63180	WO200061629-A1	Immune/Hematopoietic, Reproductive
HE8FC45	B64422	WO200077255-A1	Cancer
HETCI16	B64423	WO200077255-A1	Cancer
HFCBL53	B64427	WO200077255-A1	Cancer
HE6GR29	B64429	WO200077255-A1	Cancer
HBJEA15	B64432	WO200077255-A1	Cancer
HAPNJ33	B64434	WO200077255-A1	Cancer
HTXKB57	B64435	WO200077255-A1	Cancer
HDLAL94	B64438	WO200077255-A1	Cancer
HFVHX08	B64440	WO200077255-A1	Cancer
HMVCS92	B64441	WO200077255-A1	Cancer
HNEDQ02	B64443	WO200077255-A1	Cancer
HPWTF53	B64450	WO200077255-A1	Cancer
HRTAP63	B64451	WO200077255-A1	Cancer
HASAU26	B64453	WO200077255-A1	Cancer
HPWTF23	B64456	WO200077255-A1	Cancer
HSLHG78	B64457	WO200077255-A1	Cancer
HTNBJ15	B64461	WO200077255-A1	Cancer
HTXJW06	B64463	WO200077255-A1	Cancer
HUKFV37	B64466	WO200077255-A1	Cancer
HWBBU75	B64468	WO200077255-A1	Cancer
HWBEF34	B64469	WO200077255-A1	Immune/Hematopoietic, Neural/Sensory
HDLAL94	B64492	WO200077255-A1	Cancer
HUKFV37	B64539	WO200077255-A1	Cancer
HUKFV37	B64540	WO200077255-A1	Cancer
HMSGU30	B64549	WO200077197-A1	Cancer
HMSHU20	B64550	WO200077197-A1	Immune/Hematopoietic, Reproductive
HMTAB77	B64551	WO200077197-A1	Cancer
HMUBX48	B64552	WO200077197-A1	Musculoskeletal, Reproductive
HMWCG28	B64553	WO200077197-A1	Cancer
HMWFO89	B64555	WO200077197-A1	Cancer
HMWGM41	B64556	WO200077197-A1	Cancer
HMWGV85	B64557	WO200077197-A1	Cancer

HNDAC35	B64558	WO200077197-A1	Cancer
HNGDN07	B64559	WO200077197-A1	Immune/Hematopoietic, Reproductive
HOFMF63	B64564	WO200077197-A1	Cancer
HOSEO83	B64567	WO200077197-A1	Cancer
HPIAL55	B64568	WO200077197-A1	Cancer
HRAAF59	B64569	WO200077197-A1	Excretory
HSDIK31	B64571	WO200077197-A1	Cancer
HSDJG47	B64573	WO200077197-A1	Cancer
HSOAT44	B64574	WO200077197-A1	Cancer
HTAEF02	B64578	WO200077197-A1	Immune/Hematopoietic
HTLCX82	B64579	WO200077197-A1	Cancer
HADDP51	B64581	WO200077197-A1	Cancer
HAOAG15	B64584	WO200077197-A1	Cancer
HATCS79	B64585	WO200077197-A1	Endocrine, Immune/Hematopoietic
HMWGM41	B64604	WO200077197-A1	Cancer
HOFMF63	B64612	WO200077197-A1	Cancer
HEMBP72	B64627	WO200077197-A1	Cancer
HEMBP72	B64628	WO200077197-A1	Cancer
HAOAG15	B64657	WO200077197-A1	Cancer
HAOAG15	B64658	WO200077197-A1	Cancer
HKGDO12	B64667	WO200077237-A1	Cancer
HLQAD72	B64668	WO200077237-A1	Cancer
HFXHM93	B64672	WO200077237-A1	Neural/Sensory
HEBCW57	B64675	WO200077237-A1	Mixed Fetal, Neural/Sensory
HAECD28	B64676	WO200077237-A1	Cancer
HADXA10	B64677	WO200077237-A1	Cancer
HELEL76	B64678	WO200077237-A1	Cancer
HETBJ88	B64680	WO200077237-A1	Cancer
HETCM67	B64681	WO200077237-A1	Cancer
HFCBL53	B64682	WO200077237-A1	Cancer
HFEBK75	B64683	WO200077237-A1	Connective/Epithelial
HFIIZ61	B64684	WO200077237-A1	Cancer
HGBDL51	B64688	WO200077237-A1	Cancer
HGLDA95	B64689	WO200077237-A1	Cancer
HGLDB06	B64690	WO200077237-A1	Cancer
HHBEI14	B64691	WO200077237-A1	Cancer
HLDBG17	B64697	WO200077237-A1	Cancer
HMCIH27	B64701	WO200077237-A1	Cancer
HMEKW71	B64702	WO200077237-A1	Cancer
HNGEA90	B64705	WO200077237-A1	Immune/Hematopoietic
HNHEN70	B64708	WO200077237-A1	Cancer
HOAAH51	B64711	WO200077237-A1	Cancer
HORBL77	B64712	WO200077237-A1	Cancer
HODAH46	B64713	WO200077237-A1	Cancer
HMWEM23	B64718	WO200077237-A1	Cancer
HNEBX72	B64775	WO200077256-A1	Immune/Hematopoietic, Neural/Sensory
H6EEA48	B64776	WO200077256-A1	Cancer
HACBS22	B64778	WO200077256-A1	Cancer
HADDP23	B64779	WO200077256-A1	Cancer
HAGFD75	B64782	WO200077256-A1	Cancer
HAGFS57	B64783	WO200077256-A1	Cancer



HBIBX03	B65005	WO200075375-A1	Cancer
HBMVI06	B65006	WO200075375-A1	Cancer
HDPBA28	B65007	WO200075375-A1	Cancer
HDPUH26	B65008	WO200075375-A1	Cancer
HJTAD07	B65009	WO200075375-A1	Cancer
HDPPJ60	B65010	WO200075375-A1	Cancer
HLTAU74	B65011	WO200075375-A1	Cancer
HCFNN16	B65012	WO200075375-A1	Cancer
HCWUI05	B65013	WO200075375-A1	Immune/Hematopoietic
HCEBC76	B65014	WO200075375-A1	Neural/Sensory
HTEGT82	B65015	WO200075375-A1	Digestive, Reproductive
HLHTP35	B65016	WO200075375-A1	Cancer
HSYAZ63	B65017	WO200075375-A1	Cancer
HLTCR13	B65018	WO200075375-A1	Cancer
HPMCV08	B65019	WO200075375-A1	Cancer
HEGAK44	B65021	WO200075375-A1	Cancer
HEGAK44	B65022	WO200075375-A1	Cancer
HSXCV85	B65024	WO200075375-A1	Neural/Sensory, Reproductive
HAQBZ15	B65025	WO200075375-A1	Cancer
HDPMM34	R76127	WO9517092-A	Cancer
HAPBR31	R76128	WO9517092-A	Cancer
HPTXE69	R76818	WO9520398-A	Cancer
HPTGA39	R76820	WO9520398-A	Cancer
HFTDJ13	R81567	WO9606169-A1	Cancer
HPAAA47	R88481	WO9601270-A1	Cancer
HDQAC88	R93087	WO9605856-A1	Cancer
HCHBM70	W06550	WO9639419-A1	Cancer
HAPBR31	W07203	WO9634891-A1	Cancer
HDPMM34	W07204	WO9634891-A1	Cancer
HDTAX72	W07606	WO9639522-A1	Cancer
HPBCW46	W09405	WO9639158-A1	Mixed Fetal, Neural/Sensory
HE9ME29	W09408	WO9639486-A1	Cancer
HDTAX72	W10574	WO9624668-A1	Cancer
HGCOP28	W12692	WO9639424-A1	Cancer
HDQAC88	W22670	WO9731098-A1	Cancer
HDPJJ70	W22732	WO9724929-A1	Cancer
HSKHZ53	W23663	WO9729189-A1	Cancer
HATCY89	W27087	WO9725349-A1	Cancer
HNTCF82	W27224	WO9735870-A1	Cardiovascular, Connective/Epithelial, Reproductive
HFEBJ25	W29291	WO9735010-A1	Cancer
HKMMP34	W29292	WO9735010-A1	Cancer
HBMBJ94	W31527	WO9737022-A1	Digestive, Immune/Hematopoietic
HDPVA94	W31902	WO9737021-A1	Cancer
HFIZF58	W32110	WO9738012-A1	Cancer
HSLDE91	W32112	WO9734998-A1	Cancer
HDPVA94	W32323	WO9736915-A1	Cancer
HETFO52	W35803	WO9734997-A1	Neural/Sensory, Reproductive
HETEZ10	W35804	WO9734997-A1	Cancer
HBMTO47	W35904	WO9738003-A1	Cancer

HDQDX59	W37002	WO9733902-A1	Cancer
H2LAJ93	W37844	WO9807749-A1	Cancer
HMWIP18	W37845	WO9807749-A1	Cancer
HMQDN51	W37935	WO9808870-A1	Cancer
HOECW07	W37946	WO9821236-A1	Cancer
HTHBJ48	W41938	WO9748807-A1	Digestive, Immune/Hematopoietic
HCHBM70	W46882	US5733748-A	Cancer
HAHSD23	W48334	WO9807881-A1	Cancer
HTEHH47	W48335	WO9807754-A1	Cancer
HPASD50	W48391	WO9807735-A1	Cancer
HDQMC88	W52842	WO9807862-A2	Connective/Epithelial, Immune/Hematopoietic
HKACN58	W53897	WO9808969-A1	Cancer
HOEAL47	W57635	WO9812344-A1	Cancer
HAPBR31	W57697	WO9814582-A1	Cancer
HDPMM34	W57698	WO9814582-A1	Cancer
HE9ME29	W58704	US5780263-A	Cancer
HDTAX72	W58901	WO9814477-A1	Cancer
HISCH47	W61621	WO9831799-A2	Cancer
HDPIR89	W61622	WO9831799-A2	Digestive, Immune/Hematopoietic
HAIDQ59	W61623	WO9831799-A2	Cancer
HHFEK40	W61624	WO9831799-A2	Cancer
HGBGV89	W61625	WO9831799-A2	Digestive
HUVBB80	W61626	WO9831799-A2	Cancer
HFGAG96	W61629	WO9831799-A2	Cancer
HDTEA84	W63622	WO9830694-A2	Cancer
HPRCB54	W64668	WO9830693-A2	Cancer
HAGFY16	W67808	WO9842738-A1	Cancer
HASAV70	W67811	WO9842738-A1	Cancer
HBNAF22	W67812	WO9842738-A1	Cancer
HCDDR90	W67814	WO9842738-A1	Cancer
HCEEF50	W67815	WO9842738-A1	Cardiovascular, Neural/Sensory
HCEMU42	W67816	WO9842738-A1	Cancer
HCENE16	W67817	WO9842738-A1	Cancer
HMSJJ74	W67818	WO9842738-A1	Cancer
HCUBF15	W67819	WO9842738-A1	Immune/Hematopoietic
HE2DE47	W67820	WO9842738-A1	Cancer
HKMLH01	W67821	WO9842738-A1	Cancer
HE9DG49	W67822	WO9842738-A1	Cancer
HELBA06	W67823	WO9842738-A1	Cancer
HSLFM29	W67824	WO9842738-A1	Cancer
HELBW38	W67825	WO9842738-A1	Cancer
HFEAF41	W67828	WO9842738-A1	Connective/Epithelial, Digestive
HFTBE43	W67831	WO9842738-A1	Cancer
HLHSV96	W67835	WO9842738-A1	Respiratory
HLTBX31	W67837	WO9842738-A1	Cancer
HLTCJ63	W67838	WO9842738-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HMQAJ64	W67840	WO9842738-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive

HOABG65	W67841	WO9842738-A1	Musculoskeletal
HODCL36	W67842	WO9842738-A1	Cancer
HODCL50	W67843	WO9842738-A1	Reproductive
HODCZ16	W67845	WO9842738-A1	Cancer
HTOEU03	W67846	WO9842738-A1	Cancer
HPBCJ74	W67847	WO9842738-A1	Cancer
HSIDQ18	W67850	WO9842738-A1	Cancer
HSJBQ79	W67852	WO9842738-A1	Cancer
HTEJN13	W67854	WO9842738-A1	Neural/Sensory, Reproductive
HTHBL86	W67855	WO9842738-A1	Immune/Hematopoietic
HTSFO71	W67856	WO9842738-A1	Cancer
HAPNO80	W67857	WO9842738-A1	Cancer
HBIBZ09	W67858	WO9842738-A1	Cancer
HCFLD84	W67859	WO9842738-A1	Cancer
HE8EZ48	W67861	WO9842738-A1	Cancer
HEBGF73	W67862	WO9842738-A1	Cancer
HFEBF41	W67863	WO9842738-A1	Cancer
HFRBU14	W67864	WO9842738-A1	Neural/Sensory
HHGCO88	W67867	WO9842738-A1	Cancer
HHGDB72	W67869	WO9842738-A1	Cancer
HHGDI71	W67870	WO9842738-A1	Excretory
HHSDI45	W67871	WO9842738-A1	Cancer
HHSEB66	W67872	WO9842738-A1	Cancer
HAUAI83	W67873	WO9842738-A1	Reproductive
HKDBL30	W67874	WO9842738-A1	Cancer
HLDBQ19	W67875	WO9842738-A1	Cancer
HMSGT42	W67876	WO9842738-A1	Cancer
HMWIC78	W67877	WO9842738-A1	Cancer
HMWIR31	W67878	WO9842738-A1	Cancer
HNTAC73	W67880	WO9842738-A1	Cancer
HOSEI45	W67881	WO9842738-A1	Cancer
HOSFD58	W67882	WO9842738-A1	Cancer
HSAUM95	W67883	WO9842738-A1	Cancer
HSAUR67	W67884	WO9842738-A1	Immune/Hematopoietic
HSKDI81	W67885	WO9842738-A1	Cancer
HOUFJ08	W67886	WO9842738-A1	Cancer
HTLEX50	W67887	WO9842738-A1	Cancer
HSKHL65	W67888	WO9842738-A1	Cancer
HHFGA11	W67889	WO9842738-A1	Cancer
HAQCF47	W67890	WO9842738-A1	Cancer
HBXFG80	W67891	WO9842738-A1	Cancer
HFLQB16	W67895	WO9842738-A1	Cancer
HBMCP89	W67896	WO9842738-A1	Cancer
HE6DG34	W67897	WO9842738-A1	Cancer
HE9DG49	W67898	WO9842738-A1	Cancer
HELBA06	W67899	WO9842738-A1	Cancer
HMQAJ64	W67900	WO9842738-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HODCL36	W67901	WO9842738-A1	Cancer
HODCL36	W67902	WO9842738-A1	Cancer
HCMSD80	W67903	WO9842738-A1	Cancer
HPBCJ74	W67904	WO9842738-A1	Cancer
HHGDU04	W67905	WO9842738-A1	Cancer

HTEJN13	W67907	WO9842738-A1	Neural/Sensory, Reproductive
HAUCC47	W67909	WO9842738-A1	Cancer
HOSFD58	W67913	WO9842738-A1	Cancer
HSKHL65	W67916	WO9842738-A1	Cancer
HHFGA11	W67917	WO9842738-A1	Cancer
HOEBX83	W67918	WO9842738-A1	Cancer
HHFGA11	W67919	WO9842738-A1	Cancer
HTSFO71	W67967	WO9842738-A1	Cancer
HKFBC53	W68002	WO9842738-A1	Cancer
HSPBS71	W69221	WO9828420-A1	Connective/Epithelial, Digestive, Immune/Hematopoietic
HDPBT77	W69232	WO9831806-A2	Digestive, Immune/Hematopoietic, Reproductive
HNFFL83	W69233	WO9831806-A2	Digestive, Immune/Hematopoietic
HETHE81	W71593	WO9833912-A1	Cancer
HSVBZ80	W73397	WO9854206-A1	Cancer
HTAAU21	W73398	WO9854206-A1	Cancer
HUSIR91	W73400	WO9854206-A1	Cancer
HADMC21	W73401	WO9854206-A1	Cancer
HAGFM45	W73402	WO9854206-A1	Cancer
HAIBE65	W73403	WO9854206-A1	Cancer
HAQBH57	W73404	WO9854206-A1	Cancer
HATCX80	W73405	WO9854206-A1	Cancer
HLDOT61	W73408	WO9854206-A1	Cancer
HEMCM42	W73409	WO9854206-A1	Cancer
HFCDW34	W73411	WO9854206-A1	Cancer
HTTEU91	W73412	WO9854206-A1	Cancer
HHGBF89	W73413	WO9854206-A1	Mixed Fetal
HKMLN27	W73415	WO9854206-A1	Cancer
HLYAZ61	W73419	WO9854206-A1	Immune/Hematopoietic
HMQDT36	W73420	WO9854206-A1	Cancer
HETFI51	W73428	WO9854206-A1	Cancer
HUSIR91	W73429	WO9854206-A1	Cancer
HHGBF89	W73430	WO9854206-A1	Mixed Fetal
HPWBA10	W73432	WO9854206-A1	Immune/Hematopoietic, Reproductive
HPMBQ91	W74413	EP892053-A2	Reproductive
HBGBW52	W74732	WO9839448-A2	Cancer
HCUFQ22	W74734	WO9839448-A2	Immune/Hematopoietic
HLDOU93	W74738	WO9839448-A2	Digestive, Musculoskeletal, Reproductive
HNGJJ68	W74741	WO9839448-A2	Cancer
HCFAW04	W74742	WO9839448-A2	Immune/Hematopoietic
HLMAV65	W74743	WO9839448-A2	Cancer
HPMFD84	W74744	WO9839448-A2	Cancer
HE6DB26	W74745	WO9839448-A2	Cancer
HODBD33	W74747	WO9839448-A2	Reproductive
HBJAE44	W74750	WO9839448-A2	Immune/Hematopoietic
HCFME41	W74751	WO9839448-A2	Cancer
HOGCO71	W74752	WO9839448-A2	Cancer
HOSEX08	W74753	WO9839448-A2	Cancer

HSKNJ72	W74754	WO9839448-A2	Digestive, Musculoskeletal
HEBEB69	W74755	WO9839448-A2	Neural/Sensory, Reproductive
HE6EH18	W74756	WO9839448-A2	Mixed Fetal, Neural/Sensory
HSSDM73	W74758	WO9839448-A2	Musculoskeletal, Neural/Sensory, Reproductive
HMKCU94	W74761	WO9839448-A2	Cancer
HRDEW41	W74762	WO9839448-A2	Cancer
HBGDA21	W74764	WO9839448-A2	Cancer
HFGAK75	W74765	WO9839448-A2	Cancer
HFSAU96	W74766	WO9839448-A2	Cancer
HOVCL83	W74767	WO9839448-A2	Cancer
HBICM48	W74769	WO9839448-A2	Cancer
HLTCL35	W74770	WO9839448-A2	Cancer
HRSAN45	W74771	WO9839448-A2	Cancer
HSNBB14	W74772	WO9839448-A2	Cancer
HMABL38	W74773	WO9839448-A2	Cancer
HSKDK47	W74774	WO9839448-A2	Cancer
HOSFH03	W74775	WO9839448-A2	Cancer
HOGAV75	W74776	WO9839448-A2	Cancer
HBXDO23	W74777	WO9839448-A2	Cancer
HAGBI17	W74778	WO9839448-A2	Cancer
HPRCA31	W74780	WO9839448-A2	Cancer
HPRCE95	W74781	WO9839448-A2	Cancer
HHTLC66	W74782	WO9839448-A2	Cancer
HMAJ02	W74783	WO9839448-A2	Cancer
HPRCU93	W74784	WO9839448-A2	Cancer
HSAXS65	W74785	WO9839448-A2	Cancer
HHFHN61	W74787	WO9839448-A2	Cancer
HCWEF90	W74788	WO9839448-A2	Cancer
HFRAU10	W74790	WO9839448-A2	Neural/Sensory
HATDT67	W74791	WO9839448-A2	Cancer
HOUBG93	W74792	WO9839448-A2	Cancer
HMWEX24	W74793	WO9839448-A2	Cancer
HTOCD52	W74795	WO9839448-A2	Digestive, Immune/Hematopoietic, Reproductive
HTGCP16	W74796	WO9839448-A2	Cancer
HKIXR69	W74797	WO9839448-A2	Cancer
HE6CN34	W74800	WO9839448-A2	Cancer
HSQEL25	W74802	WO9839448-A2	Cancer
HEBEG68	W74803	WO9839448-A2	Cancer
HBIAB39	W74804	WO9839448-A2	Cancer
HOEAS24	W74805	WO9839448-A2	Cancer
HETDD75	W74806	WO9839448-A2	Cancer
HSKNE46	W74807	WO9839448-A2	Cancer
HPMFL27	W74808	WO9839448-A2	Cancer
HPRAX55	W74810	WO9839448-A2	Cancer
HE2PL77	W74812	WO9839448-A2	Cancer
HLHAU92	W74813	WO9839448-A2	Cancer
HTPEG42	W74814	WO9839448-A2	Cancer
HAUAV32	W74816	WO9839448-A2	Cancer
HNEBI60	W74817	WO9839448-A2	Cancer



HTSEL31	W74819	WO9839448-A2	Cancer
HAUBL57	W74820	WO9839448-A2	Cancer
HE6CT48	W74822	WO9839448-A2	Digestive, Mixed Fetal
HMDAA61	W74823	WO9839448-A2	Cancer
HAQBK61	W74824	WO9839448-A2	Cancer
HAQBF73	W74825	WO9839448-A2	Cancer
HAQBT94	W74826	WO9839448-A2	Cancer
HLQAB52	W74828	WO9839448-A2	Cancer
HE2BG03	W74830	WO9839448-A2	Cancer
HCUBC79	W74832	WO9839448-A2	Cancer
HSVAF07	W74833	WO9839448-A2	Cancer
HT3AM65	W74834	WO9839448-A2	Cancer
HE6DK18	W74835	WO9839448-A2	Cancer
HEBEK93	W74836	WO9839448-A2	Cancer
HJPCM10	W74837	WO9839448-A2	Cancer
HSXBL78	W74838	WO9839448-A2	Cancer
HOEAW81	W74839	WO9839448-A2	Cancer
HEAAR60	W74841	WO9839448-A2	Cancer
HOVBA03	W74843	WO9839448-A2	Cancer
HGBGK76	W74844	WO9839448-A2	Digestive, Neural/Sensory
HBMUW78	W74845	WO9839448-A2	Cancer
HATCM76	W74848	WO9839448-A2	Cancer
H6EBJ64	W74849	WO9839448-A2	Cancer
HDDAD77	W74850	WO9839448-A2	Cancer
HSPAG15	W74853	WO9839448-A2	Cancer
HUSHH48	W74855	WO9839448-A2	Cancer
HHSCV65	W74857	WO9839448-A2	Cancer
HHSDQ41	W74858	WO9839448-A2	Cancer
HEBFU93	W74860	WO9839448-A2	Excretory, Neural/Sensory, Reproductive
HSGSC60	W74861	WO9839448-A2	Cancer
HPMGD24	W74862	WO9839448-A2	Cancer
HPTVC60	W74863	WO9839448-A2	Cancer
HSKNE18	W74864	WO9839448-A2	Cancer
HMWIF35	W74865	WO9839448-A2	Cancer
HMWGI25	W74866	WO9839448-A2	Cancer
HSKGF03	W74867	WO9839448-A2	Cancer
HMSKE75	W74868	WO9839448-A2	Cancer
HCM SH30	W74869	WO9839448-A2	Cancer
HTWCB92	W74870	WO9839448-A2	Cancer
HBMDM46	W74871	WO9839448-A2	Cancer
HFXHL79	W74873	WO9839448-A2	Cancer
HBJFJ73	W74874	WO9839448-A2	Cancer
HSJAP03	W74875	WO9839448-A2	Cancer
H6EAD09	W74876	WO9839448-A2	Cancer
HTLEF62	W74879	WO9839448-A2	Cancer
HTLAD94	W74880	WO9839448-A2	Cancer
HTSFQ12	W74881	WO9839448-A2	Cancer
HCE2K05	W74882	WO9839448-A2	Cancer
HLTED27	W74884	WO9839448-A2	Cancer
HMKBA64	W74885	WO9839448-A2	Cancer
HNFCO49	W74886	WO9839448-A2	Cancer

HCELB21	W74887	WO9839448-A2	Cancer
HSAAS44	W74889	WO9839448-A2	Cancer
HAFAL73	W74890	WO9839448-A2	Cancer
HSAWF26	W74891	WO9839448-A2	Digestive, Immune/Hematopoietic, Musculoskeletal
HMQDN51	W74892	WO9839448-A2	Cancer
H2LAO11	W74894	WO9839448-A2	Cancer
HPTTU11	W74896	WO9839448-A2	Cancer
HTEDJ34	W74898	WO9839448-A2	Cancer
HFTAR26	W74900	WO9839448-A2	Cancer
H2MBF44	W74901	WO9839448-A2	Cancer
HE8BI92	W74902	WO9839448-A2	Cancer
HFTBR48	W74903	WO9839448-A2	Cancer
HE9CM64	W74904	WO9839448-A2	Cancer
HATAV51	W74905	WO9839448-A2	Cancer
HCEEK08	W74907	WO9839448-A2	Cancer
HAFAU18	W74908	WO9839448-A2	Cancer
HETBY74	W74909	WO9839448-A2	Cancer
HTOAF35	W74910	WO9839448-A2	Cancer
HCRBX32	W74911	WO9839448-A2	Cancer
HEBGB80	W74912	WO9839448-A2	Cancer
HFAMH74	W74913	WO9839448-A2	Cancer
HLMAV65	W74920	WO9839448-A2	Cancer
HMAGF23	W74922	WO9839448-A2	Cancer
HE6EH18	W74929	WO9839448-A2	Mixed Fetal, Neural/Sensory
HMKCU94	W74930	WO9839448-A2	Cancer
HBGDA21	W74931	WO9839448-A2	Cancer
HFKFN58	W74932	WO9839448-A2	Cancer
HSNBB14	W74935	WO9839448-A2	Cancer
HOSFH03	W74937	WO9839448-A2	Cancer
HAGBI17	W74939	WO9839448-A2	Cancer
HPRCA31	W74940	WO9839448-A2	Cancer
HPRCU93	W74943	WO9839448-A2	Cancer
HPDDK44	W74944	WO9839448-A2	Cancer
HCWEF90	W74946	WO9839448-A2	Cancer
HFRAU10	W74947	WO9839448-A2	Neural/Sensory
HBIAB39	W74953	WO9839448-A2	Cancer
HBIAB39	W74954	WO9839448-A2	Cancer
HOEAS24	W74955	WO9839448-A2	Cancer
HOEAS24	W74956	WO9839448-A2	Cancer
HPRAX55	W74958	WO9839448-A2	Cancer
HTPEG42	W74960	WO9839448-A2	Cancer
HAUAV32	W74961	WO9839448-A2	Cancer
HNEBI60	W74962	WO9839448-A2	Cancer
HAUBL57	W74963	WO9839448-A2	Cancer
HAUBL57	W74964	WO9839448-A2	Cancer
HE6CT48	W74965	WO9839448-A2	Digestive, Mixed Fetal
HMDAA61	W74966	WO9839448-A2	Cancer
HAQBK61	W74967	WO9839448-A2	Cancer
HCUHB01	W74968	WO9839448-A2	Cancer
HETHE07	W74970	WO9839448-A2	Cancer
HETHE07	W74971	WO9839448-A2	Cancer

HLQAB52	W74972	WO9839448-A2	Cancer
HEONN58	W74973	WO9839448-A2	Cancer
HIBEK16	W74974	WO9839448-A2	Cancer
HE2BG03	W74975	WO9839448-A2	Cancer
HCUBC79	W74976	WO9839448-A2	Cancer
HSVAF07	W74978	WO9839448-A2	Cancer
HSVAF07	W74979	WO9839448-A2	Cancer
HT3AM65	W74980	WO9839448-A2	Cancer
HT3AM65	W74981	WO9839448-A2	Cancer
HJPCM10	W74983	WO9839448-A2	Cancer
HJPCM10	W74984	WO9839448-A2	Cancer
HOVBA03	W74987	WO9839448-A2	Cancer
H6EBJ64	W74990	WO9839448-A2	Cancer
HUSHH48	W74991	WO9839448-A2	Cancer
HEBFU93	W74992	WO9839448-A2	Excretory, Neural/Sensory, Reproductive
HPTVC60	W74993	WO9839448-A2	Cancer
HMWIF35	W74995	WO9839448-A2	Cancer
HSKGF03	W74996	WO9839448-A2	Cancer
HBJFJ73	W75000	WO9839448-A2	Cancer
HCFBC03	W75001	WO9839448-A2	Cancer
HSJAP03	W75002	WO9839448-A2	Cancer
HE6FL83	W75005	WO9839448-A2	Cancer
HPTTU11	W75013	WO9839448-A2	Cancer
H2MBF44	W75015	WO9839448-A2	Cancer
HE9CM64	W75018	WO9839448-A2	Cancer
HAFAU18	W75021	WO9839448-A2	Cancer
HSACC16	W75050	WO9839448-A2	Cancer
HGCMD20	W75057	WO9839446-A2	Cancer
HLDBG33	W75058	WO9839446-A2	Cancer
HLHEJ14	W75059	WO9839446-A2	Cancer
HKCSR70	W75060	WO9839446-A2	Cancer
HBMCY91	W75062	WO9839446-A2	Immune/Hematopoietic
HSSGE07	W75063	WO9839446-A2	Cancer
HBMBX59	W75064	WO9839446-A2	Immune/Hematopoietic, Reproductive
HNGIT22	W75065	WO9839446-A2	Immune/Hematopoietic
HERAD57	W75066	WO9839446-A2	Connective/Epithelial
HCENJ40	W75067	WO9839446-A2	Cancer
HCSRA90	W75068	WO9839446-A2	Cardiovascular, Musculoskeletal
HBJFC03	W75069	WO9839446-A2	Immune/Hematopoietic
HTEBY26	W75071	WO9839446-A2	Cancer
HMABH07	W75072	WO9839446-A2	Cancer
HSKNY94	W75073	WO9839446-A2	Cancer
HMCDA67	W75074	WO9839446-A2	Immune/Hematopoietic
HOSFF45	W75075	WO9839446-A2	Cancer
HMJAA51	W75076	WO9839446-A2	Cancer
HTEBF05	W75077	WO9839446-A2	Reproductive
HTEAL31	W75078	WO9839446-A2	Cancer
HSKXE91	W75080	WO9839446-A2	Cancer
HPWTB39	W75081	WO9839446-A2	Mixed Fetal, Reproductive
HTLEV12	W75082	WO9839446-A2	Reproductive

HSPAF93	W75083	WO9839446-A2	Digestive
HHFGL62	W75084	WO9839446-A2	Cardiovascular
HCE1U14	W75085	WO9839446-A2	Cancer
HTHBA79	W75087	WO9839446-A2	Cancer
HAGBB70	W75088	WO9839446-A2	Cancer
HETDG84	W75089	WO9839446-A2	Cancer
HTEGA81	W75090	WO9839446-A2	Cancer
HTXAK60	W75091	WO9839446-A2	Cancer
HMHBN40	W75092	WO9839446-A2	Cancer
HFVGS85	W75093	WO9839446-A2	Cancer
HERAH81	W75094	WO9839446-A2	Cancer
HMSEU04	W75095	WO9839446-A2	Cancer
HNEDJ57	W75096	WO9839446-A2	Cancer
HNTME13	W75097	WO9839446-A2	Cancer
HSXBI25	W75098	WO9839446-A2	Cancer
HSXCK41	W75099	WO9839446-A2	Cancer
HE8CJ26	W75100	WO9839446-A2	Cancer
HTTDS54	W75101	WO9839446-A2	Cancer
HHFCW44	W75102	WO9839446-A2	Cancer
HMCBP63	W75103	WO9839446-A2	Cancer
HEMGE83	W75104	WO9839446-A2	Cancer
HHSDC22	W75105	WO9839446-A2	Digestive, Neural/Sensory
HHSDZ57	W75106	WO9839446-A2	Cancer
HCRBS80	W75107	WO9839446-A2	Cancer
HMMAB12	W75108	WO9839446-A2	Immune/Hematopoietic, Neural/Sensory
HSKDW02	W75109	WO9839446-A2	Cancer
HWHHL34	W75110	WO9839446-A2	Cancer
HODAZ50	W75111	WO9839446-A2	Reproductive
HCEWC82	W75112	WO9839446-A2	Cancer
HE6ES13	W75113	WO9839446-A2	Cancer
HSSEP68	W75114	WO9839446-A2	Cancer
HRDEV41	W75115	WO9839446-A2	Cancer
HILCJ01	W75116	WO9839446-A2	Cancer
HSATP28	W75117	WO9839446-A2	Cancer
HBJEM49	W75119	WO9839446-A2	Cancer
HSLDJ95	W75120	WO9839446-A2	Cancer, Immune
HSREG44	W75121	WO9839446-A2	Cancer
HTXCT40	W75122	WO9839446-A2	Cancer
HRGDF73	W75123	WO9839446-A2	Cancer
HKMND45	W75124	WO9839446-A2	Cancer
HPEBD70	W75125	WO9839446-A2	Cancer
HLMDX11	W75126	WO9839446-A2	Cancer
HKCSR70	W75128	WO9839446-A2	Cancer
HETBI87	W75129	WO9839446-A2	Reproductive
HSSGE07	W75130	WO9839446-A2	Cancer
HCENJ40	W75132	WO9839446-A2	Cancer
HSNBL85	W75135	WO9839446-A2	Cancer
HMAAD57	W75137	WO9839446-A2	Cancer
HMAAD57	W75138	WO9839446-A2	Cancer
HSKNY94	W75139	WO9839446-A2	Cancer
HOSFF45	W75140	WO9839446-A2	Cancer
HMJAA51	W75141	WO9839446-A2	Cancer
HTEAL31	W75142	WO9839446-A2	Cancer

HSPAF93	W75145	WO9839446-A2	Digestive
HHFGL62	W75146	WO9839446-A2	Cardiovascular
HTHBA79	W75148	WO9839446-A2	Cancer
HTEGA81	W75151	WO9839446-A2	Cancer
HTEGA81	W75152	WO9839446-A2	Cancer
HMHBN40	W75154	WO9839446-A2	Cancer
HLHDL62	W75155	WO9839446-A2	Cancer
HSXBI25	W75156	WO9839446-A2	Cancer
HSXCK41	W75157	WO9839446-A2	Cancer
HTTDS54	W75159	WO9839446-A2	Cancer
HHFCW44	W75160	WO9839446-A2	Cancer
HHSDZ57	W75161	WO9839446-A2	Cancer
HAICS58	W75162	WO9839446-A2	Cancer
HAICS58	W75163	WO9839446-A2	Cancer
HSKDW02	W75165	WO9839446-A2	Cancer
HETGL41	W75166	WO9839446-A2	Cancer
HODAZ50	W75167	WO9839446-A2	Reproductive
HE6ES13	W75168	WO9839446-A2	Cancer
HSSEP68	W75169	WO9839446-A2	Cancer
HRDEV41	W75171	WO9839446-A2	Cancer
HHFGL41	W75172	WO9839446-A2	Cancer
HBJEM49	W75173	WO9839446-A2	Cancer
HFTAK35	W75174	WO9839446-A2	Cancer
HTXCT40	W75175	WO9839446-A2	Cancer
HRDBF52	W75176	WO9839446-A2	Cancer
HKMND45	W75177	WO9839446-A2	Cancer
HDTBJ30	W75178	WO9839446-A2	Cancer
HLMDX11	W75179	WO9839446-A2	Cancer
HCEAB46	W75196	WO9840483-A2	Cancer
HCEDH81	W75197	WO9840483-A2	Cancer
HELDY41	W75200	WO9840483-A2	Cancer
HETDM20	W75201	WO9840483-A2	Cancer
HE2DX30	W75202	WO9840483-A2	Cancer
HJBCD89	W75204	WO9840483-A2	Cancer
HJTA17	W75205	WO9840483-A2	Cancer
HLTBS22	W75206	WO9840483-A2	Cancer
HNFCV70	W75208	WO9840483-A2	Cancer
HNFGF45	W75210	WO9840483-A2	Cancer
HOVAB12	W75211	WO9840483-A2	Cancer
HPMBQ91	W75212	WO9840483-A2	Reproductive
HRSMC69	W75214	WO9840483-A2	Cancer
HSQFP46	W75216	WO9840483-A2	Cancer
HTEAE62	W75218	WO9840483-A2	Cardiovascular, Reproductive
HTEBY11	W75219	WO9840483-A2	Reproductive
HTEEB42	W75220	WO9840483-A2	Cancer
HTPBY11	W75221	WO9840483-A2	Cancer
HCEDH81	W75224	WO9840483-A2	Cancer
HJBCD89	W75226	WO9840483-A2	Cancer
HNFCV70	W75227	WO9840483-A2	Cancer
HPMBQ91	W75228	WO9840483-A2	Reproductive
HBMSH54	W75231	WO9840483-A2	Cancer
HSDEG01	W75232	WO9840483-A2	Cancer
HSQFP46	W75233	WO9840483-A2	Cancer
HTEBY11	W75234	WO9840483-A2	Reproductive

HYACC84	W75245	WO9840483-A2	Cancer
HETAG43	W76253	WO9831818-A2	Digestive, Reproductive
HOSBI96	W78128	WO9856804-A1	Cancer
HPDDC77	W78131	WO9856804-A1	Cancer
HPEBD85	W78132	WO9856804-A1	Digestive, Reproductive
HPMGQ80	W78135	WO9856804-A1	Cancer
HSDES04	W78140	WO9856804-A1	Cancer
HSHBQ68	W78141	WO9856804-A1	Cancer
HSKBO20	W78142	WO9856804-A1	Cancer
HSKZE52	W78145	WO9856804-A1	Cancer
HWTAZ75	W78146	WO9856804-A1	Cancer
HSVAG05	W78148	WO9856804-A1	Cancer
HSVBF78	W78149	WO9856804-A1	Cancer
HSXBO51	W78150	WO9856804-A1	Cancer
HT4AI54	W78152	WO9856804-A1	Cancer
HTEHU93	W78153	WO9856804-A1	Reproductive
HMSDG61	W78154	WO9856804-A1	Cancer
HTLDQ11	W78157	WO9856804-A1	Reproductive
HTOBX52	W78158	WO9856804-A1	Cancer
HTTCN24	W78159	WO9856804-A1	Cancer
HTXCS21	W78160	WO9856804-A1	Cancer
HBMBB80	W78164	WO9856804-A1	Digestive, Immune/Hematopoietic
HSXBP68	W78166	WO9856804-A1	Cancer
HFFAT33	W78167	WO9856804-A1	Cancer
HFGAG96	W78168	WO9856804-A1	Cancer
HETFJ05	W78169	WO9856804-A1	Cancer
HE8BX01	W78170	WO9856804-A1	Cancer
HMSJU68	W78171	WO9856804-A1	Cancer
HOSCZ41	W78172	WO9856804-A1	Cancer
HSQEA85	W78174	WO9856804-A1	Cancer
HSTAG52	W78175	WO9856804-A1	Cancer
HBXGP76	W78177	WO9856804-A1	Immune/Hematopoietic, Neural/Sensory
HE6GL64	W78178	WO9856804-A1	Cardiovascular, Immune/Hematopoietic, Mixed Fetal
HESAL35	W78179	WO9856804-A1	Connective/Epithelial, Mixed Fetal
HNHAL34	W78183	WO9856804-A1	Cancer
HOSFF78	W78184	WO9856804-A1	Cancer
HPMCC16	W78188	WO9856804-A1	Cancer
HOUQC17	W78189	WO9856804-A1	Cancer
HTOFC34	W78192	WO9856804-A1	Cancer
H2CBJ08	W78193	WO9856804-A1	Cancer
HAGFT48	W78194	WO9856804-A1	Cancer
HCE5M29	W78195	WO9856804-A1	Cancer
HCFNN01	W78197	WO9856804-A1	Digestive, Immune/Hematopoietic, Neural/Sensory
HE7TF86	W78198	WO9856804-A1	Cancer
HHGAU81	W78200	WO9856804-A1	Cancer
HPTRF90	W78206	WO9856804-A1	Cancer
HSRDH01	W78207	WO9856804-A1	Cancer

HSAWD74	W78208	WO9856804-A1	Cancer
HTEJO12	W78209	WO9856804-A1	Digestive, Reproductive
HTLAB43	W78210	WO9856804-A1	Cancer
HTWCT03	W78211	WO9856804-A1	Immune/Hematopoietic
HSDES04	W78213	WO9856804-A1	Cancer
HT3BE24	W78214	WO9856804-A1	Cancer
HTTCN24	W78216	WO9856804-A1	Cancer
HCRAZ77	W78221	WO9856804-A1	Cancer
HFGAG96	W78222	WO9856804-A1	Cancer
HADTN61	W78223	WO9856804-A1	Cancer
HLYBF81	W78224	WO9856804-A1	Cancer
HSTBE27	W78225	WO9856804-A1	Cancer
HMSDG61	W78263	WO9856804-A1	Cancer
HTOBX52	W78274	WO9856804-A1	Cancer
HFGAG96	W78295	WO9856804-A1	Cancer
HCE5M29	W78316	WO9856804-A1	Cancer
HLCAA05	W78321	WO9856804-A1	Cancer
HTLEF68	W78326	WO9856804-A1	Cancer
HSJAR34	W79739	WO9846746-A1	Cancer
HOUCQ17	W80285	EP874050-A2	Cancer
HCWHZ93	W83931	WO9845712-A2	Immune/Hematopoietic, Neural/Sensory
HE2FV03	W83933	WO9845712-A2	Cancer
HCDAG36	W83934	WO9845712-A2	Cancer
HMQBU44	W83935	WO9845712-A2	Cancer
HLHCM89	W83938	WO9845712-A2	Cancer
HLHEF26	W83939	WO9845712-A2	Cancer
HLHEO50	W83940	WO9845712-A2	Cancer
HDSAE10	W83941	WO9845712-A2	Cancer
HSKNK73	W83942	WO9845712-A2	Cancer
HSSMS41	W83943	WO9845712-A2	Cancer
HNGBV36	W83944	WO9845712-A2	Cancer
HNGDE27	W83945	WO9845712-A2	Immune/Hematopoietic
HPFDU90	W83947	WO9845712-A2	Cancer
HRLMD77	W83948	WO9845712-A2	Cancer
HRLMF92	W83949	WO9845712-A2	Cancer
HLHDZ58	W88535	WO9854963-A2	Respiratory
HLMMJ13	W88536	WO9854963-A2	Immune/Hematopoietic, Musculoskeletal, Reproductive
HNFED65	W88539	WO9854963-A2	Excretory, Immune/Hematopoietic
HNHDX07	W88540	WO9854963-A2	Immune/Hematopoietic
HNHGC82	W88541	WO9854963-A2	Immune/Hematopoietic
HNHGO09	W88542	WO9854963-A2	Immune/Hematopoietic
HOUBE18	W88543	WO9854963-A2	Cancer
HOUDL69	W88544	WO9854963-A2	Cancer
HPMFI71	W88545	WO9854963-A2	Cancer
HPTBB03	W88548	WO9854963-A2	Cancer
HPTWA66	W88549	WO9854963-A2	Cancer
HPTWC08	W88550	WO9854963-A2	Cancer
HRGCZ46	W88551	WO9854963-A2	Cancer
HSAVU34	W88552	WO9854963-A2	Cancer
HSDFW61	W88553	WO9854963-A2	Cancer

HSQEO84	W88556	WO9854963-A2	Cancer
HSXAM05	W88557	WO9854963-A2	Cancer
HSXAS67	W88558	WO9854963-A2	Neural/Sensory
HTDAF28	W88559	WO9854963-A2	Cancer
HTOAM21	W88562	WO9854963-A2	Immune/Hematopoietic
HETCH46	W88563	WO9854963-A2	Cancer
HJPCD40	W88564	WO9854963-A2	Cancer
HTWBY48	W88565	WO9854963-A2	Immune/Hematopoietic
HWTBF59	W88568	WO9854963-A2	Cancer
HAGFB60	W88570	WO9854963-A2	Neural/Sensory
HATEF60	W88571	WO9854963-A2	Cancer
HCDAR68	W88573	WO9854963-A2	Cancer
HMDAN54	W88575	WO9854963-A2	Immune/Hematopoietic, Neural/Sensory
HCEEC15	W88577	WO9854963-A2	Cancer
HCESF40	W88578	WO9854963-A2	Immune/Hematopoietic, Neural/Sensory
HCFMV39	W88579	WO9854963-A2	Cancer
HCNAP62	W88581	WO9854963-A2	Cancer
HCUDC07	W88583	WO9854963-A2	Immune/Hematopoietic
HCWBB42	W88584	WO9854963-A2	Immune/Hematopoietic
HE9ND48	W88592	WO9854963-A2	Mixed Fetal
HEBBW11	W88593	WO9854963-A2	Cancer
HEMAE80	W88595	WO9854963-A2	Cardiovascular, Musculoskeletal, Reproductive
HFEB A88	W88596	WO9854963-A2	Cancer
HGBAJ93	W88599	WO9854963-A2	Cancer
HGBBQ69	W88600	WO9854963-A2	Cancer
HHFHJ59	W88602	WO9854963-A2	Cancer
HHPFD63	W88606	WO9854963-A2	Endocrine, Immune/Hematopoietic, Neural/Sensory
HHSEG23	W88607	WO9854963-A2	Neural/Sensory
HKIXL73	W88609	WO9854963-A2	Cancer
HKMNC43	W88610	WO9854963-A2	Excretory
HMEJE31	W88611	WO9854963-A2	Cardiovascular
HNFAE54	W88613	WO9854963-A2	Cancer
HNFIJH45	W88614	WO9854963-A2	Immune/Hematopoietic
HNGBT31	W88615	WO9854963-A2	Immune/Hematopoietic
HNGIN60	W88616	WO9854963-A2	Immune/Hematopoietic, Neural/Sensory
HNHDW42	W88618	WO9854963-A2	Immune/Hematopoietic
HNHFL57	W88619	WO9854963-A2	Immune/Hematopoietic
HOGAR52	W88620	WO9854963-A2	Cancer
HOSBZ55	W88621	WO9854963-A2	Cancer
HOSDI92	W88622	WO9854963-A2	Cancer
HPBCU51	W88623	WO9854963-A2	Cancer
HNTSU23	W88628	WO9854963-A2	Cancer
HRDFB85	W88629	WO9854963-A2	Cancer
HSKGN81	W88631	WO9854963-A2	Cancer
HSPAH56	W88632	WO9854963-A2	Cancer
HDTAL71	W88633	WO9854963-A2	Cancer
HSXCS62	W88634	WO9854963-A2	Cancer
HTEKM35	W88636	WO9854963-A2	Neural/Sensory, Reproductive



HTGEP89	W88637	WO9854963-A2	Immune/Hematopoietic, Neural/Sensory
HTPCN79	W88640	WO9854963-A2	Digestive, Neural/Sensory
HTSGM54	W88641	WO9854963-A2	Cancer
HTWAF58	W88643	WO9854963-A2	Immune/Hematopoietic
HTWBY29	W88644	WO9854963-A2	Cancer
HUKFC71	W88645	WO9854963-A2	Cancer
HCE2V74	W88646	WO9854963-A2	Cancer
HFXBW82	W88652	WO9854963-A2	Neural/Sensory
HIBED17	W88654	WO9854963-A2	Cancer
HPMCJ92	W88657	WO9854963-A2	Musculoskeletal, Reproductive
HPWAZ95	W88658	WO9854963-A2	Reproductive
HSUBW09	W88660	WO9854963-A2	Digestive, Immune/Hematopoietic
HALSQ59	W88666	WO9854963-A2	Cancer
HAIBP89	W88667	WO9854963-A2	Cancer
HBXGK12	W88669	WO9854963-A2	Cancer
HFKFJ07	W88670	WO9854963-A2	Cancer
HCWHZ24	W88672	WO9854963-A2	Immune/Hematopoietic
HE2GT20	W88673	WO9854963-A2	Cancer
HFTCT67	W88676	WO9854963-A2	Cancer
HUSIT49	W88680	WO9854963-A2	Cancer
HNHED86	W88684	WO9854963-A2	Immune/Hematopoietic
HNHFQ63	W88686	WO9854963-A2	Immune/Hematopoietic
HAGDQ47	W88692	WO9854963-A2	Cancer
HAICP19	W88693	WO9854963-A2	Cancer
HCEQA68	W88699	WO9854963-A2	Neural/Sensory
HCFNF11	W88701	WO9854963-A2	Cancer
HCRBL20	W88702	WO9854963-A2	Cancer
HDSAP81	W88704	WO9854963-A2	Cancer
HE2CT29	W88705	WO9854963-A2	Mixed Fetal
HE8MG65	W88706	WO9854963-A2	Cancer
HE9FB42	W88707	WO9854963-A2	Cancer
HEMAM41	W88708	WO9854963-A2	Cancer
HEMCV19	W88709	WO9854963-A2	Cancer
HETAR54	W88711	WO9854963-A2	Cancer
HETBX14	W88712	WO9854963-A2	Cancer
HFKFI40	W88714	WO9854963-A2	Cancer
HFXHN68	W88715	WO9854963-A2	Cancer
HGBFO79	W88716	WO9854963-A2	Cancer
HGLAM56	W88717	WO9854963-A2	Cancer
HHLBA89	W88718	WO9854963-A2	Digestive
HIASB53	W88723	WO9854963-A2	Cancer
HJABZ65	W88724	WO9854963-A2	Cancer
HJPBB39	W88725	WO9854963-A2	Cancer
HLHSK94	W88726	WO9854963-A2	Cancer
HLMIW92	W88728	WO9854963-A2	Cancer
HLTDB65	W88730	WO9854963-A2	Cancer
HNFAH08	W88733	WO9854963-A2	Cancer
HNGBE45	W88735	WO9854963-A2	Immune/Hematopoietic, Reproductive
HNHCM59	W88737	WO9854963-A2	Cancer
HCDEO95	W88740	WO9854963-A2	Immune/Hematopoietic, Musculoskeletal,

			Reproductive
HLMMJ13	W88741	WO9854963-A2	Immune/Hematopoietic, Musculoskeletal, Reproductive
HPTWA66	W88742	WO9854963-A2	Cancer
HSVU34	W88743	WO9854963-A2	Cancer
HSQEO84	W88744	WO9854963-A2	Cancer
HETCH46	W88745	WO9854963-A2	Cancer
HWTBF59	W88746	WO9854963-A2	Cancer
HCESF40	W88747	WO9854963-A2	Immune/Hematopoietic, Neural/Sensory
HOFNZ45	W88748	WO9854963-A2	Reproductive
HPWAN23	W88749	WO9854963-A2	Cancer
HCRBL20	W88754	WO9854963-A2	Cancer
HE8MG65	W88755	WO9854963-A2	Cancer
HEMAM41	W88756	WO9854963-A2	Cancer
HSVU34	W88760	WO9854963-A2	Cancer
HFHDN80	W88824	WO9854963-A2	Cardiovascular, Digestive, Immune/Hematopoietic
HHFHR32	W88830	WO9854963-A2	Cancer
HSKCP69	W89024	WO9854963-A2	Cancer
HTLCU04	W89076	WO9854963-A2	Cancer
HSKHZ53	W92460	US5871969-A	Cancer
HFIZH13	W94466	WO9900415-A1	Cancer
HE9SF68	W97350	WO9903982-A1	Cancer
HTECE94	Y00258	WO9906423-A1	Cancer
HTWAH05	Y00259	WO9906423-A1	Cancer
HAQAN31	Y00260	WO9906423-A1	Cancer
HAUAQ39	Y00261	WO9906423-A1	Cancer
HBNAU27	Y00262	WO9906423-A1	Cancer
HSIDD28	Y00263	WO9906423-A1	Cancer
HCABR41	Y00264	WO9906423-A1	Cancer
HCUAQ30	Y00265	WO9906423-A1	Immune/Hematopoietic
HE2AF21	Y00266	WO9906423-A1	Mixed Fetal
HE2DC87	Y00267	WO9906423-A1	Mixed Fetal
HE2PO86	Y00269	WO9906423-A1	Cancer
HFCBD73	Y00272	WO9906423-A1	Cancer
HSVAJ05	Y00273	WO9906423-A1	Cancer
HLHSA86	Y00274	WO9906423-A1	Cancer
H2CAA57	Y00278	WO9906423-A1	Cancer
HADFV30	Y00279	WO9906423-A1	Cancer
HAIBO71	Y00280	WO9906423-A1	Connective/Epithelial, Digestive, Immune/Hematopoietic
HAPAT76	Y00281	WO9906423-A1	Cancer
HLHEB47	Y00282	WO9906423-A1	Cancer
HLHEF54	Y00283	WO9906423-A1	Cancer
HLMMJ78	Y00286	WO9906423-A1	Immune/Hematopoietic
HLQBQ85	Y00287	WO9906423-A1	Cancer
HLQBR11	Y00288	WO9906423-A1	Cancer
HLWBZ56	Y00289	WO9906423-A1	Cancer
HMCAR20	Y00292	WO9906423-A1	Cancer
HMCV55	Y00293	WO9906423-A1	Immune/Hematopoietic
HMEFS61	Y00294	WO9906423-A1	Cardiovascular
HMEJY78	Y00295	WO9906423-A1	Cancer

HMWHH16	Y00298	WO9906423-A1	Immune/Hematopoietic
HNFFC27	Y00300	WO9906423-A1	Immune/Hematopoietic
HNFFC39	Y00301	WO9906423-A1	Immune/Hematopoietic, Reproductive
HNGAM20	Y00302	WO9906423-A1	Immune/Hematopoietic
HNGDS53	Y00304	WO9906423-A1	Immune/Hematopoietic
HNGEW13	Y00307	WO9906423-A1	Immune/Hematopoietic
HNGEY51	Y00308	WO9906423-A1	Immune/Hematopoietic
HNGEZ47	Y00309	WO9906423-A1	Immune/Hematopoietic
HNGFQ33	Y00310	WO9906423-A1	Immune/Hematopoietic
HNGFU38	Y00311	WO9906423-A1	Immune/Hematopoietic
HSKXE22	Y00313	WO9906423-A1	Cancer
HNHBE49	Y00314	WO9906423-A1	Immune/Hematopoietic
HNHEC59	Y00315	WO9906423-A1	Immune/Hematopoietic
HNHEI54	Y00317	WO9906423-A1	Immune/Hematopoietic, Reproductive
HNHER77	Y00318	WO9906423-A1	Immune/Hematopoietic
HNHES40	Y00319	WO9906423-A1	Immune/Hematopoietic
HNHEV43	Y00320	WO9906423-A1	Immune/Hematopoietic
HNHFL46	Y00321	WO9906423-A1	Immune/Hematopoietic
HNHFP80	Y00322	WO9906423-A1	Immune/Hematopoietic
HNHFS63	Y00323	WO9906423-A1	Immune/Hematopoietic
HNHGC56	Y00324	WO9906423-A1	Immune/Hematopoietic
HRDEL61	Y00328	WO9906423-A1	Musculoskeletal
HSAUC38	Y00329	WO9906423-A1	Immune/Hematopoietic
HSAUF49	Y00330	WO9906423-A1	Immune/Hematopoietic
HSAUK57	Y00331	WO9906423-A1	Immune/Hematopoietic
HSAUL82	Y00332	WO9906423-A1	Immune/Hematopoietic
HSAXI90	Y00333	WO9906423-A1	Immune/Hematopoietic
HSDGW43	Y00335	WO9906423-A1	Neural/Sensory
HSDJM31	Y00336	WO9906423-A1	Digestive, Neural/Sensory
HSDJR23	Y00337	WO9906423-A1	Digestive, Neural/Sensory
HSDMA90	Y00338	WO9906423-A1	Digestive, Endocrine, Neural/Sensory
HSVAJ05	Y00340	WO9906423-A1	Cancer
HAPAT76	Y00341	WO9906423-A1	Cancer
HNGAM20	Y00344	WO9906423-A1	Immune/Hematopoietic
HTXBK30	Y01135	WO9901020-A2	Cancer
H2MBB56	Y01136	WO9901020-A2	Cancer
HIBCW32	Y01138	WO9901020-A2	Cancer
HLHCI58	Y01139	WO9901020-A2	Cancer
HLMFG37	Y01140	WO9901020-A2	Cancer
HBCAO31	Y01141	WO9901020-A2	Cancer
HRDDR94	Y01142	WO9901020-A2	Cancer
HSIDY06	Y01143	WO9901020-A2	Cancer
HSKGO49	Y01144	WO9901020-A2	Cancer
HBXGM67	Y01146	WO9901020-A2	Neural/Sensory
HUFAC36	Y01147	WO9901020-A2	Cancer
HAGBZ81	Y01148	WO9901020-A2	Excretory, Neural/Sensory
HBJCK69	Y01150	WO9901020-A2	Immune/Hematopoietic
HCACJ81	Y01152	WO9901020-A2	Cancer
HBMWP47	Y01154	WO9901020-A2	Cancer

HIBCW32	Y01155	WO9901020-A2	Cancer
HCACJ81	Y01158	WO9901020-A2	Cancer
HCE3F11	Y01206	WO9901020-A2	Digestive, Neural/Sensory
HSXBV35	Y01383	WO9903990-A1	Neural/Sensory
HTGAW51	Y01385	WO9903990-A1	Immune/Hematopoietic
HTEGM07	Y01387	WO9903990-A1	Cancer
HTWFK09	Y01389	WO9903990-A1	Immune/Hematopoietic
HTXDJ88	Y01390	WO9903990-A1	Immune/Hematopoietic
HUSGC54	Y01391	WO9903990-A1	Cardiovascular, Immune/Hematopoietic, Neural/Sensory
HWTAD49	Y01392	WO9903990-A1	Cancer
HWTBK81	Y01393	WO9903990-A1	Cancer
HACBH16	Y01394	WO9903990-A1	Connective/Epithelial
HCUDE16	Y01395	WO9903990-A1	Cancer
HLWBZ73	Y01397	WO9903990-A1	Cancer
HNGFR75	Y01398	WO9903990-A1	Immune/Hematopoietic
HNHFO29	Y01400	WO9903990-A1	Immune/Hematopoietic
HONAH29	Y01401	WO9903990-A1	Cancer
HGCAB62	Y01402	WO9903990-A1	Cancer
HAQBI01	Y01403	WO9903990-A1	Cancer
HDPBA48	Y01405	WO9903990-A1	Immune/Hematopoietic
HE6CT22	Y01406	WO9903990-A1	Mixed Fetal, Reproductive
HE6CT56	Y01407	WO9903990-A1	Mixed Fetal, Neural/Sensory
HE6CY88	Y01408	WO9903990-A1	Mixed Fetal
HE9FT63	Y01409	WO9903990-A1	Cancer
HE9ND43	Y01410	WO9903990-A1	Digestive, Mixed Fetal, Neural/Sensory
HERAN63	Y01411	WO9903990-A1	Connective/Epithelial, Reproductive
HHBAG14	Y01413	WO9903990-A1	Cancer
HMAU73	Y01417	WO9903990-A1	Cancer
HMEAI74	Y01418	WO9903990-A1	Cancer
HPMBZ15	Y01421	WO9903990-A1	Cancer
HROAE16	Y01422	WO9903990-A1	Cancer
HSAYM40	Y01423	WO9903990-A1	Immune/Hematopoietic
HTBAB28	Y01426	WO9903990-A1	Immune/Hematopoietic
HAQBT52	Y01428	WO9903990-A1	Cancer
HBIBL04	Y01429	WO9903990-A1	Cancer
HBJCI95	Y01430	WO9903990-A1	Cancer
HBNBQ61	Y01431	WO9903990-A1	Reproductive
HE2ID06	Y01432	WO9903990-A1	Cancer
HEBCM63	Y01433	WO9903990-A1	Cancer
HFFAK76	Y01434	WO9903990-A1	Neural/Sensory
HFRBF28	Y01435	WO9903990-A1	Neural/Sensory
HGBHM89	Y01436	WO9903990-A1	Cancer
HLMBP18	Y01437	WO9903990-A1	Immune/Hematopoietic
HAGFG63	Y01439	WO9903990-A1	Cancer
HODAZ55	Y01440	WO9903990-A1	Reproductive
HODDF08	Y01441	WO9903990-A1	Reproductive
HOSDK95	Y01442	WO9903990-A1	Musculoskeletal
HOUAR65	Y01443	WO9903990-A1	Connective/Epithelial

HSVAC77	Y01444	WO9903990-A1	Cancer
HRSMC69	Y01445	WO9903990-A1	Cancer
HNECF34	Y01446	WO9903990-A1	Immune/Hematopoietic
HAQAI46	Y01447	WO9903990-A1	Cancer
HAQBI01	Y01448	WO9903990-A1	Cancer
HJAAT30	Y01453	WO9903990-A1	Cancer
HPMJ158	Y01458	WO9903990-A1	Cancer
HNECF34	Y01477	WO9903990-A1	Immune/Hematopoietic
HCEIA77	Y02650	WO9902546-A1	Cancer
HCFCE10	Y02651	WO9902546-A1	Immune/Hematopoietic
HCHAA63	Y02653	WO9902546-A1	Cancer
HCNSP40	Y02654	WO9902546-A1	Cancer
HDAAC10	Y02655	WO9902546-A1	Cardiovascular, Digestive, Reproductive
HE8CV18	Y02656	WO9902546-A1	Cancer
HFGAL10	Y02659	WO9902546-A1	Mixed Fetal, Neural/Sensory, Reproductive
HFKEB72	Y02660	WO9902546-A1	Excretory, Reproductive
HFTCU19	Y02661	WO9902546-A1	Cancer
HFXHN31	Y02662	WO9902546-A1	Neural/Sensory
HCEND31	Y02663	WO9902546-A1	Cancer
HJABB94	Y02664	WO9902546-A1	Cancer
HLTAI94	Y02666	WO9902546-A1	Immune/Hematopoietic, Reproductive
HMELR03	Y02668	WO9902546-A1	Cardiovascular, Immune/Hematopoietic, Mixed Fetal
HMKAH10	Y02669	WO9902546-A1	Neural/Sensory, Reproductive
HMKCW19	Y02670	WO9902546-A1	Cancer
HMSJW18	Y02671	WO9902546-A1	Cancer
HMWGY01	Y02672	WO9902546-A1	Immune/Hematopoietic
HNFID82	Y02673	WO9902546-A1	Immune/Hematopoietic
HNFIG36	Y02674	WO9902546-A1	Immune/Hematopoietic
HNGEV29	Y02675	WO9902546-A1	Immune/Hematopoietic
HNGJJ65	Y02677	WO9902546-A1	Immune/Hematopoietic
HSLBF69	Y02687	WO9902546-A1	Immune/Hematopoietic, Musculoskeletal, Reproductive
HSVBH58	Y02689	WO9902546-A1	Cancer
HTADX17	Y02692	WO9902546-A1	Immune/Hematopoietic, Reproductive
HTDAD22	Y02693	WO9902546-A1	Cancer
HTEDS39	Y02694	WO9902546-A1	Cancer
HTEHH53	Y02695	WO9902546-A1	Reproductive
HTLDP69	Y02696	WO9902546-A1	Cancer
HTPCS60	Y02698	WO9902546-A1	Cancer
HUKBH05	Y02699	WO9902546-A1	Cancer
HADFK68	Y02703	WO9902546-A1	Connective/Epithelial
HADGG19	Y02704	WO9902546-A1	Connective/Epithelial, Musculoskeletal
HAEAV45	Y02705	WO9902546-A1	Cardiovascular, Reproductive

HARAA15	Y02706	WO9902546-A1	Neural/Sensory
HBAFQ54	Y02708	WO9902546-A1	Cancer
HBIAS26	Y02710	WO9902546-A1	Cancer
HBJFU48	Y02711	WO9902546-A1	Immune/Hematopoietic
HBJFV28	Y02712	WO9902546-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HBMWB01	Y02713	WO9902546-A1	Immune/Hematopoietic
HBMXN79	Y02714	WO9902546-A1	Cancer
HBMXP84	Y02715	WO9902546-A1	Cancer
HCFMM26	Y02716	WO9902546-A1	Immune/Hematopoietic
HCNAV36	Y02717	WO9902546-A1	Cancer
HCNSB01	Y02718	WO9902546-A1	Cancer
HCRBR74	Y02719	WO9902546-A1	Cancer
HCUBN59	Y02720	WO9902546-A1	Immune/Hematopoietic
HCUDB38	Y02721	WO9902546-A1	Immune/Hematopoietic
HCUFZ62	Y02722	WO9902546-A1	Immune/Hematopoietic
HDPCO25	Y02724	WO9902546-A1	Immune/Hematopoietic
HDPHI51	Y02725	WO9902546-A1	Immune/Hematopoietic
HE9FE83	Y02727	WO9902546-A1	Immune/Hematopoietic, Mixed Fetal, Musculoskeletal
HFPDE69	Y02731	WO9902546-A1	Neural/Sensory
HGBGV89	Y02732	WO9902546-A1	Digestive
HGLDE38	Y02733	WO9902546-A1	Cancer
HHGDU58	Y02734	WO9902546-A1	Musculoskeletal
HHTLF25	Y02735	WO9902546-A1	Cancer
HKAFB88	Y02737	WO9902546-A1	Cancer
HLHFP03	Y02738	WO9902546-A1	Respiratory
HLYAF80	Y02741	WO9902546-A1	Immune/Hematopoietic
HMKDD07	Y02743	WO9902546-A1	Immune/Hematopoietic, Neural/Sensory
HMKDS08	Y02744	WO9902546-A1	Excretory, Neural/Sensory
HMSHM14	Y02745	WO9902546-A1	Immune/Hematopoietic
HMWDC28	Y02746	WO9902546-A1	Cancer
HNFIU96	Y02749	WO9902546-A1	Immune/Hematopoietic
HNGAX58	Y02751	WO9902546-A1	Immune/Hematopoietic
HNHDL85	Y02754	WO9902546-A1	Immune/Hematopoietic
HNHFU59	Y02755	WO9902546-A1	Immune/Hematopoietic
HNHFW22	Y02756	WO9902546-A1	Immune/Hematopoietic
HODCJ90	Y02758	WO9902546-A1	Cancer
HPEBT80	Y02760	WO9902546-A1	Reproductive
HSDAG05	Y02761	WO9902546-A1	Cancer
HSDGR57	Y02762	WO9902546-A1	Cancer
HSDJJ82	Y02763	WO9902546-A1	Neural/Sensory
HSDZM95	Y02764	WO9902546-A1	Cancer
HSKYU29	Y02766	WO9902546-A1	Cancer
HSNAA55	Y02767	WO9902546-A1	Cancer
HSQFP66	Y02768	WO9902546-A1	Excretory, Neural/Sensory
HJPBB94	Y02769	WO9902546-A1	Cancer
HSSJN64	Y02770	WO9902546-A1	Musculoskeletal
HSVAQ28	Y02771	WO9902546-A1	Cancer
HFTCU19	Y02775	WO9902546-A1	Cancer
HGLAM53	Y02777	WO9902546-A1	Immune/Hematopoietic,

			Neural/Sensory, Reproductive
HMKCW19	Y02778	WO9902546-A1	Cancer
HMWGY01	Y02779	WO9902546-A1	Immune/Hematopoietic
HSAH66	Y02782	WO9902546-A1	Digestive
HUKEX85	Y02785	WO9902546-A1	Musculoskeletal, Reproductive
HSIDI15	Y02975	WO9902546-A1	Digestive, Immune/Hematopoietic
HUKEJ46	Y03850	WO9909198-A1	Digestive, Reproductive
HPASD50	Y04120	WO9909161-A1	Cancer
HPASD50	Y04121	WO9909161-A1	Cancer
HSDIT06	Y04295	WO9910363-A1	Neural/Sensory, Reproductive
HSKEI54	Y04297	WO9910363-A1	Cancer
HTNAG39	Y04300	WO9910363-A1	Cancer
HTODL90	Y04301	WO9910363-A1	Immune/Hematopoietic
HTWDC20	Y04302	WO9910363-A1	Immune/Hematopoietic
HUFAT34	Y04303	WO9910363-A1	Cancer
HAICJ23	Y04305	WO9910363-A1	Cancer
HAPOF67	Y04306	WO9910363-A1	Digestive, Excretory, Musculoskeletal
HE8DG53	Y04308	WO9910363-A1	Cancer
HFSAY85	Y04309	WO9910363-A1	Cancer
HHEDD41	Y04310	WO9910363-A1	Cancer
HKCSO46	Y04311	WO9910363-A1	Cancer
HKGAV60	Y04312	WO9910363-A1	Cancer
HKGDJ66	Y04314	WO9910363-A1	Cancer
HMC DK27	Y04315	WO9910363-A1	Cancer
HMC DX48	Y04316	WO9910363-A1	Cancer
HMIAS24	Y04317	WO9910363-A1	Immune/Hematopoietic, Neural/Sensory
HNFE G11	Y04318	WO9910363-A1	Immune/Hematopoietic
HNGEP09	Y04319	WO9910363-A1	Immune/Hematopoietic
HTXKK52	Y04320	WO9910363-A1	Immune/Hematopoietic
HNGJP90	Y04321	WO9910363-A1	Immune/Hematopoietic
HFVIF40	Y06461	WO9931116-A1	Cancer
HFCCQ50	Y06462	WO9931116-A1	Cancer
HDPIE88	Y06511	WO9936565-A1	Cancer
HCWHN10	Y07746	WO9909155-A1	Immune/Hematopoietic
HDTAE40	Y07748	WO9909155-A1	Digestive, Immune/Hematopoietic
HE8DY08	Y07751	WO9909155-A1	Cancer
HE9ND27	Y07753	WO9909155-A1	Cancer
HCE3G69	Y07754	WO9909155-A1	Cancer
HEAAX57	Y07755	WO9909155-A1	Reproductive
HEMGD15	Y07759	WO9909155-A1	Cancer
HEQBR95	Y07760	WO9909155-A1	Cancer
HFKGE44	Y07764	WO9909155-A1	Cancer
HFP CY39	Y07765	WO9909155-A1	Cancer
HFXDX75	Y07768	WO9909155-A1	Neural/Sensory
HFXJC53	Y07770	WO9909155-A1	Neural/Sensory, Reproductive, Respiratory

HFXJW48	Y07771	WO9909155-A1	Cancer
HGBGO11	Y07772	WO9909155-A1	Cancer
HGBHM10	Y07773	WO9909155-A1	Cancer
HSWAY58	Y07776	WO9909155-A1	Cancer
HTEIM65	Y07779	WO9909155-A1	Immune/Hematopoietic, Reproductive
HTHBX95	Y07780	WO9909155-A1	Cancer
HTLDQ56	Y07781	WO9909155-A1	Reproductive
HTOFU06	Y07782	WO9909155-A1	Immune/Hematopoietic, Musculoskeletal
HTWEE31	Y07785	WO9909155-A1	Immune/Hematopoietic
HUSAO56	Y07789	WO9909155-A1	Cancer
HUSIJ08	Y07790	WO9909155-A1	Cancer
HAGBD57	Y07791	WO9909155-A1	Excretory, Neural/Sensory
HBAFA04	Y07793	WO9909155-A1	Cancer
HBJES16	Y07794	WO9909155-A1	Cancer
HCEFZ05	Y07796	WO9909155-A1	Mixed Fetal, Neural/Sensory
HCFMX95	Y07797	WO9909155-A1	Immune/Hematopoietic
HLYHA71	Y07798	WO9909155-A1	Cancer
HEBAL06	Y07800	WO9909155-A1	Neural/Sensory
HEIAB33	Y07801	WO9909155-A1	Cancer
HEPBC02	Y07802	WO9909155-A1	Cancer
HFTBY96	Y07803	WO9909155-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HKMMM61	Y07804	WO9909155-A1	Cancer
HLQBQ38	Y07806	WO9909155-A1	Cancer
HMKCP66	Y07807	WO9909155-A1	Neural/Sensory
HWTAL40	Y07808	WO9909155-A1	Cancer
HNHDR03	Y07809	WO9909155-A1	Immune/Hematopoietic
HNHFH41	Y07810	WO9909155-A1	Immune/Hematopoietic
HNHFI81	Y07811	WO9909155-A1	Immune/Hematopoietic
HOSFQ28	Y07812	WO9909155-A1	Cancer
HPRAL78	Y07813	WO9909155-A1	Cancer
HEAAA85	Y07814	WO9909155-A1	Cancer
HDTAR09	Y07816	WO9909155-A1	Cancer
HLYHA71	Y07843	WO9909155-A1	Cancer
HCWCH14	Y07852	WO9918208-A1	Immune/Hematopoietic
HE9MI43	Y07855	WO9918208-A1	Cancer
HE2PI29	Y07859	WO9918208-A1	Cancer
HLHDP83	Y07862	WO9918208-A1	Cancer
HSIAS17	Y07863	WO9918208-A1	Cancer
HOSDG32	Y07866	WO9918208-A1	Cancer
HMUBU59	Y07867	WO9918208-A1	Cancer
HWTCE21	Y07868	WO9918208-A1	Cancer
HFIUM15	Y07869	WO9918208-A1	Cancer
HTLAF13	Y07872	WO9918208-A1	Reproductive
HTLFI93	Y07873	WO9918208-A1	Immune/Hematopoietic, Reproductive, Respiratory
HBXGI20	Y07874	WO9918208-A1	Cancer
HTPBH21	Y07875	WO9918208-A1	Connective/Epithelial, Digestive, Reproductive



HSQAB87	Y07876	WO9918208-A1	Cancer
HTEDJ94	Y07877	WO9918208-A1	Cancer
HKMLM11	Y07878	WO9918208-A1	Cancer
HNEAC05	Y07879	WO9918208-A1	Immune/Hematopoietic
HETEW02	Y07880	WO9918208-A1	Cancer
HLMCA59	Y07882	WO9918208-A1	Immune/Hematopoietic
HOAAC90	Y07883	WO9918208-A1	Musculoskeletal
HMEJQ68	Y07884	WO9918208-A1	Cancer
HRTAE58	Y07888	WO9918208-A1	Digestive, Reproductive
HSKNB54	Y07889	WO9918208-A1	Cancer
HSKNT34	Y07890	WO9918208-A1	Cancer
HTEDY42	Y07891	WO9918208-A1	Reproductive
HTLAA40	Y07892	WO9918208-A1	Reproductive
HTNBO91	Y07893	WO9918208-A1	Cancer
H6BSD90	Y07894	WO9918208-A1	Cancer
HBJBQ35	Y07895	WO9918208-A1	Immune/Hematopoietic
HCEIQ89	Y07896	WO9918208-A1	Immune/Hematopoietic, Neural/Sensory
HCNSB61	Y07897	WO9918208-A1	Digestive, Immune/Hematopoietic
HCDBO20	Y07898	WO9918208-A1	Musculoskeletal, Respiratory
HBNAW17	Y07899	WO9918208-A1	Reproductive
HEAAH81	Y07902	WO9918208-A1	Cancer
HEBAE88	Y07903	WO9918208-A1	Immune/Hematopoietic, Neural/Sensory
HFXGV31	Y07904	WO9918208-A1	Neural/Sensory
HEAAJ57	Y07905	WO9918208-A1	Immune/Hematopoietic, Reproductive
HCFMV71	Y07906	WO9918208-A1	Immune/Hematopoietic
HGBDL30	Y07910	WO9918208-A1	Digestive
HFKEN81	Y07911	WO9918208-A1	Excretory, Neural/Sensory
HFPCX36	Y07912	WO9918208-A1	Neural/Sensory
HFRAN90	Y07913	WO9918208-A1	Neural/Sensory
HHGBO91	Y07915	WO9918208-A1	Digestive, Reproductive
HERAN54	Y07917	WO9918208-A1	Connective/Epithelial
HFXDE67	Y07918	WO9918208-A1	Neural/Sensory
HFFAD59	Y07921	WO9918208-A1	Neural/Sensory
HMDAE65	Y07923	WO9918208-A1	Neural/Sensory
HMEGF92	Y07925	WO9918208-A1	Cardiovascular
HNGIK36	Y07926	WO9918208-A1	Immune/Hematopoietic
HMEJJ27	Y07927	WO9918208-A1	Cardiovascular
HNHCY64	Y07928	WO9918208-A1	Immune/Hematopoietic
HNHCY94	Y07929	WO9918208-A1	Immune/Hematopoietic
HNEBN76	Y07930	WO9918208-A1	Immune/Hematopoietic, Reproductive, Respiratory
HMEFT54	Y07931	WO9918208-A1	Cardiovascular, Musculoskeletal, Reproductive
HLQBE09	Y07932	WO9918208-A1	Digestive
HMWBC11	Y07933	WO9918208-A1	Immune/Hematopoietic
HNGJR78	Y07934	WO9918208-A1	Immune/Hematopoietic

HNGDP26	Y07935	WO9918208-A1	Immune/Hematopoietic
HNGJH63	Y07936	WO9918208-A1	Immune/Hematopoietic
HMDAL04	Y07937	WO9918208-A1	Neural/Sensory
HMWHX28	Y07938	WO9918208-A1	Immune/Hematopoietic
HNHGB09	Y07942	WO9918208-A1	Immune/Hematopoietic
HNHHA15	Y07943	WO9918208-A1	Immune/Hematopoietic
HHGDC01	Y07944	WO9918208-A1	Cancer
HMWGU74	Y07945	WO9918208-A1	Immune/Hematopoietic
HNGCF72	Y07946	WO9918208-A1	Immune/Hematopoietic
HOACB38	Y07947	WO9918208-A1	Musculoskeletal
HLMFD11	Y07950	WO9918208-A1	Immune/Hematopoietic
HLYBA22	Y07952	WO9918208-A1	Immune/Hematopoietic
HCWCH14	Y07953	WO9918208-A1	Immune/Hematopoietic
HBMWF85	Y10797	WO9907891-A1	Immune/Hematopoietic
HCDEJ37	Y10798	WO9907891-A1	Immune/Hematopoietic, Musculoskeletal
HCE3L18	Y10799	WO9907891-A1	Neural/Sensory
HCYBI42	Y10800	WO9907891-A1	Cancer
HE6FB81	Y10801	WO9907891-A1	Mixed Fetal
HFAMB72	Y10802	WO9907891-A1	Cancer
HFCDW42	Y10803	WO9907891-A1	Cancer
HFPAE26	Y10804	WO9907891-A1	Neural/Sensory
HFXJM91	Y10805	WO9907891-A1	Cancer
HJABX32	Y10807	WO9907891-A1	Cancer
HJMBW30	Y10808	WO9907891-A1	Cancer
HSVAT02	Y10810	WO9907891-A1	Cancer
HSVBM90	Y10811	WO9907891-A1	Cancer
HSYBL17	Y10812	WO9907891-A1	Cancer
HTEBI28	Y10813	WO9907891-A1	Reproductive
HTPDS14	Y10814	WO9907891-A1	Cancer
HTSGG36	Y10815	WO9907891-A1	Cancer
HODCJ27	Y10816	WO9907891-A1	Cancer
HTXDB52	Y10819	WO9907891-A1	Immune/Hematopoietic, Musculoskeletal
HTXDP60	Y10820	WO9907891-A1	Cancer
HTXEB42	Y10821	WO9907891-A1	Cancer
HBAFZ29	Y10824	WO9907891-A1	Cancer
HBAHA77	Y10826	WO9907891-A1	Cancer
HBJEW84	Y10827	WO9907891-A1	Immune/Hematopoietic
HBJFE12	Y10828	WO9907891-A1	Immune/Hematopoietic
HCFBM53	Y10830	WO9907891-A1	Cancer
HCFBQ81	Y10831	WO9907891-A1	Immune/Hematopoietic
HCFCI07	Y10832	WO9907891-A1	Immune/Hematopoietic
HCFDD76	Y10833	WO9907891-A1	Cancer
HCFMJ81	Y10834	WO9907891-A1	Cancer
HCFQG45	Y10835	WO9907891-A1	Cancer
HCUBN71	Y10836	WO9907891-A1	Immune/Hematopoietic, Reproductive
HHEMA75	Y10837	WO9907891-A1	Cancer
HHPTJ65	Y10839	WO9907891-A1	Cardiovascular, Musculoskeletal, Neural/Sensory
HHSDR11	Y10840	WO9907891-A1	Neural/Sensory
HLJDQ62	Y10842	WO9907891-A1	Cancer
HKGBS49	Y10843	WO9907891-A1	Reproductive

HKISA27	Y10844	WO9907891-A1	Cancer
HKIXE06	Y10845	WO9907891-A1	Cancer
HKMMV77	Y10846	WO9907891-A1	Excretory, Reproductive
HLYAB80	Y10850	WO9907891-A1	Cancer
HLYAG19	Y10851	WO9907891-A1	Digestive, Immune/Hematopoietic
HLYBY48	Y10852	WO9907891-A1	Immune/Hematopoietic
HMUAW28	Y10853	WO9907891-A1	Immune/Hematopoietic, Musculoskeletal
HMWHC36	Y10854	WO9907891-A1	Cancer
HNFIS82	Y10856	WO9907891-A1	Digestive, Immune/Hematopoietic, Reproductive
HNGBO16	Y10859	WO9907891-A1	Immune/Hematopoietic
HNGBQ90	Y10860	WO9907891-A1	Cancer
HNGBV72	Y10861	WO9907891-A1	Immune/Hematopoietic
HNGEG08	Y10863	WO9907891-A1	Immune/Hematopoietic
HNGFI02	Y10864	WO9907891-A1	Immune/Hematopoietic
HNGGF85	Y10865	WO9907891-A1	Immune/Hematopoietic
HNGHM75	Y10866	WO9907891-A1	Immune/Hematopoietic
HNGIN84	Y10867	WO9907891-A1	Digestive, Endocrine, Immune/Hematopoietic
HNGJH08	Y10869	WO9907891-A1	Immune/Hematopoietic
HNHAH01	Y10870	WO9907891-A1	Immune/Hematopoietic
HNHET53	Y10871	WO9907891-A1	Immune/Hematopoietic
HOABP21	Y10872	WO9907891-A1	Cancer
HODAA12	Y10873	WO9907891-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HFKDH44	Y10874	WO9907891-A1	Cancer
HOVAP06	Y10875	WO9907891-A1	Reproductive
HPEAE34	Y10876	WO9907891-A1	Reproductive
HPTRO86	Y10877	WO9907891-A1	Cancer
HSAXJ60	Y10878	WO9907891-A1	Immune/Hematopoietic
HSAXM32	Y10879	WO9907891-A1	Cancer
HSKND71	Y10882	WO9907891-A1	Mixed Fetal, Musculoskeletal, Neural/Sensory
HSOAC84	Y10883	WO9907891-A1	Digestive
HFKCF34	Y10884	WO9907891-A1	Cancer
HSAAO30	Y12916	WO9911293-A1	Cancer
HSQBL21	Y12917	WO9911293-A1	Cancer
HTEFU41	Y12919	WO9911293-A1	Immune/Hematopoietic, Reproductive
HDPSP54	Y12920	WO9911293-A1	Cancer
HELFAQ07	Y12921	WO9911293-A1	Cancer
HBSAJ16	Y12923	WO9911293-A1	Connective/Epithelial, Musculoskeletal, Reproductive
HCEOC41	Y12924	WO9911293-A1	Cancer
HCUEO60	Y12926	WO9911293-A1	Immune/Hematopoietic
HDHEB60	Y12927	WO9911293-A1	Cancer
HE6AJ31	Y12928	WO9911293-A1	Mixed Fetal
HFCED59	Y12929	WO9911293-A1	Immune/Hematopoietic,

			Neural/Sensory
HFXKJ03	Y12931	WO9911293-A1	Cardiovascular, Immune/Hematopoietic, Neural/Sensory
HHFDG44	Y12932	WO9911293-A1	Cardiovascular, Endocrine, Immune/Hematopoietic
HJACG02	Y12933	WO9911293-A1	Digestive, Immune/Hematopoietic
HKGJ54	Y12934	WO9911293-A1	Cancer
HKMAB92	Y12935	WO9911293-A1	Cancer
HLMFC54	Y12937	WO9911293-A1	Immune/Hematopoietic
HLWBZ21	Y12939	WO9911293-A1	Immune/Hematopoietic, Reproductive
HMJAX71	Y12940	WO9911293-A1	Neural/Sensory
HNECU95	Y12941	WO9911293-A1	Connective/Epithelial, Immune/Hematopoietic
HNFC41	Y12942	WO9911293-A1	Cancer
HNFHD08	Y12943	WO9911293-A1	Cancer
HNGEW65	Y12944	WO9911293-A1	Endocrine, Immune/Hematopoietic
HNHEN68	Y12946	WO9911293-A1	Immune/Hematopoietic
HNHFG05	Y12947	WO9911293-A1	Immune/Hematopoietic
HODBF19	Y12948	WO9911293-A1	Cancer
HOEBK34	Y12949	WO9911293-A1	Digestive, Musculoskeletal
HPBCC51	Y12950	WO9911293-A1	Cancer
HRGDC48	Y12951	WO9911293-A1	Immune/Hematopoietic, Musculoskeletal
HSDJB13	Y12952	WO9911293-A1	Cancer
HTEHR24	Y12953	WO9911293-A1	Cancer
HARAO51	Y12957	WO9911293-A1	Cancer
HATAA15	Y12958	WO9911293-A1	Cancer
HATCK44	Y12959	WO9911293-A1	Cancer
HBIAE26	Y12960	WO9911293-A1	Neural/Sensory, Reproductive
HBMXG32	Y12961	WO9911293-A1	Immune/Hematopoietic
HCDAT43	Y12963	WO9911293-A1	Cancer
HSLJB89	Y12964	WO9911293-A1	Cancer
HBAFC77	Y12966	WO9911293-A1	Cancer
HSAAO30	Y12969	WO9911293-A1	Cancer
HFCET92	Y14078	WO9921575-A1	Cancer
HSIDU19	Y14411	WO9919339-A1	Digestive
HPRSB76	Y14412	WO9919339-A1	Reproductive
HTEIL66	Y14413	WO9919339-A1	Reproductive
HSABG21	Y14415	WO9919339-A1	Cancer
HSAXB32	Y14416	WO9919339-A1	Immune/Hematopoietic
HPEAD48	Y14417	WO9919339-A1	Reproductive
HPVAB94	Y14418	WO9919339-A1	Reproductive
HSAXB81	Y14419	WO9919339-A1	Immune/Hematopoietic
HSLCU73	Y14421	WO9919339-A1	Musculoskeletal
HTEIP36	Y14423	WO9919339-A1	Reproductive
HYBAY77	Y14424	WO9919339-A1	Immune/Hematopoietic, Musculoskeletal, Reproductive
HROAE78	Y14425	WO9919339-A1	Digestive

HSAVP17	Y14426	WO9919339-A1	Immune/Hematopoietic
HSIEA14	Y14427	WO9919339-A1	Digestive
HPEAD79	Y14430	WO9919339-A1	Reproductive
HRDED19	Y14431	WO9919339-A1	Musculoskeletal
HSAYS89	Y14432	WO9919339-A1	Immune/Hematopoietic
HTODK73	Y14433	WO9919339-A1	Cancer
HSVAM10	Y14434	WO9919339-A1	Cancer
HSPAA60	Y14439	WO9919339-A1	Digestive
HFAEF57	Y14440	WO9919339-A1	Neural/Sensory
HEGAH43	Y14441	WO9919339-A1	Digestive, Reproductive
HNGBX63	Y14443	WO9919339-A1	Immune/Hematopoietic
HE2AG50	Y14444	WO9919339-A1	Digestive, Mixed Fetal, Neural/Sensory
HCUIN80	Y14445	WO9919339-A1	Immune/Hematopoietic
HADCL29	Y14446	WO9919339-A1	Connective/Epithelial
HAPPS89	Y14447	WO9919339-A1	Cancer
HFGAH44	Y14448	WO9919339-A1	Cancer
HFIHZ96	Y14449	WO9919339-A1	Musculoskeletal
HFIUR10	Y14450	WO9919339-A1	Digestive, Immune/Hematopoietic, Musculoskeletal
HLDNA86	Y14451	WO9919339-A1	Cancer
HCUIO20	Y14453	WO9919339-A1	Immune/Hematopoietic
HLTEF12	Y14454	WO9919339-A1	Cancer
HCFBJ91	Y14455	WO9919339-A1	Immune/Hematopoietic
HHFHP90	Y14456	WO9919339-A1	Cardiovascular
HLYCQ48	Y14457	WO9919339-A1	Immune/Hematopoietic
HHLAB07	Y14458	WO9919339-A1	Digestive, Immune/Hematopoietic
HFOXEX30	Y14459	WO9919339-A1	Musculoskeletal
HBJEL68	Y14460	WO9919339-A1	Immune/Hematopoietic, Neural/Sensory
HFIUR35	Y14462	WO9919339-A1	Musculoskeletal
HFIZF58	Y16587	US5916769-A	Cancer
HNGDJ72	Y19443	WO9922243-A1	Immune/Hematopoietic
HNGEO29	Y19444	WO9922243-A1	Immune/Hematopoietic
HNHDL95	Y19445	WO9922243-A1	Immune/Hematopoietic
HAGDS35	Y19446	WO9922243-A1	Cancer
HNGEQ48	Y19447	WO9922243-A1	Immune/Hematopoietic
HNGDG40	Y19448	WO9922243-A1	Immune/Hematopoietic
HNGEN81	Y19449	WO9922243-A1	Immune/Hematopoietic
H2MAC30	Y19450	WO9922243-A1	Cancer
HNHFB16	Y19451	WO9922243-A1	Immune/Hematopoietic
HPFCL43	Y19452	WO9922243-A1	Cancer
HSATR82	Y19453	WO9922243-A1	Immune/Hematopoietic
HNHIC21	Y19455	WO9922243-A1	Immune/Hematopoietic
HOVCA92	Y19456	WO9922243-A1	Immune/Hematopoietic, Reproductive
HSDIL30	Y19458	WO9922243-A1	Neural/Sensory
HATDB65	Y19459	WO9922243-A1	Endocrine, Reproductive, Respiratory
HTTEA24	Y19461	WO9922243-A1	Digestive, Reproductive

HAGDS20	Y19462	WO9922243-A1	Neural/Sensory, Reproductive
HSDJM30	Y19463	WO9922243-A1	Digestive, Neural/Sensory
HNHEE88	Y19464	WO9922243-A1	Immune/Hematopoietic
HSLFD55	Y19465	WO9922243-A1	Musculoskeletal
HSAXJ29	Y19466	WO9922243-A1	Immune/Hematopoietic
HSFAM39	Y19467	WO9922243-A1	Reproductive
HADDZ85	Y19469	WO9922243-A1	Connective/Epithelial, Immune/Hematopoietic, Neural/Sensory
HDPCM26	Y19470	WO9922243-A1	Cancer
HSZAA13	Y19471	WO9922243-A1	Cancer
HDTBP04	Y19472	WO9922243-A1	Digestive, Immune/Hematopoietic
HHGCQ54	Y19473	WO9922243-A1	Cancer
HSNAB12	Y19474	WO9922243-A1	Cardiovascular
HBJID05	Y19475	WO9922243-A1	Immune/Hematopoietic
HSNBM49	Y19476	WO9922243-A1	Cancer
HJMBF77	Y19477	WO9922243-A1	Cancer
HJMBM38	Y19478	WO9922243-A1	Cancer
HHGCL33	Y19479	WO9922243-A1	Cancer
HCEWE20	Y19480	WO9922243-A1	Endocrine, Immune/Hematopoietic, Neural/Sensory
HCUHL13	Y19481	WO9922243-A1	Immune/Hematopoietic
HBJHO68	Y19482	WO9922243-A1	Immune/Hematopoietic
HCWDV84	Y19483	WO9922243-A1	Immune/Hematopoietic
HBXFC78	Y19484	WO9922243-A1	Cancer
HE2FI45	Y19485	WO9922243-A1	Cancer
HEOMG13	Y19486	WO9922243-A1	Digestive, Immune/Hematopoietic, Reproductive
HFAMH77	Y19487	WO9922243-A1	Cancer
HSVCF20	Y19488	WO9922243-A1	Cancer
HISAG02	Y19489	WO9922243-A1	Cancer
HCDAF84	Y19490	WO9922243-A1	Musculoskeletal
HHAAC17	Y19491	WO9922243-A1	Digestive, Musculoskeletal, Neural/Sensory
HEQAG39	Y19493	WO9922243-A1	Cancer
HKACH44	Y19494	WO9922243-A1	Cancer
HBNBG49	Y19495	WO9922243-A1	Cancer
HE2EN04	Y19496	WO9922243-A1	Cancer
HSVAA10	Y19497	WO9922243-A1	Cardiovascular
HFPBA88	Y19498	WO9922243-A1	Cancer
HHEBW54	Y19500	WO9922243-A1	Cancer
HFEBH21	Y19501	WO9922243-A1	Connective/Epithelial, Reproductive
HFTDZ36	Y19502	WO9922243-A1	Cancer
HGLAW96	Y19503	WO9922243-A1	Immune/Hematopoietic, Neural/Sensory
HKAfk41	Y19504	WO9922243-A1	Cancer
HOSEG51	Y19505	WO9922243-A1	Endocrine, Immune/Hematopoietic, Musculoskeletal



HAFBD61	Y19553	WO9922243-A1	Cancer
HBJJU28	Y19554	WO9922243-A1	Immune/Hematopoietic, Neural/Sensory
HNHEI47	Y19555	WO9922243-A1	Immune/Hematopoietic
HPMFY74	Y19556	WO9922243-A1	Reproductive
HLYAP91	Y19559	WO9922243-A1	Digestive, Immune/Hematopoietic, Reproductive
HSKNB56	Y19560	WO9922243-A1	Cancer
HHGCW91	Y19561	WO9922243-A1	Digestive, Immune/Hematopoietic
HKIYE96	Y19562	WO9922243-A1	Excretory
HLYAN59	Y19563	WO9922243-A1	Immune/Hematopoietic
HNEEE24	Y19564	WO9922243-A1	Immune/Hematopoietic
HAPRK85	Y19565	WO9922243-A1	Cancer
HLTEJ06	Y19566	WO9922243-A1	Immune/Hematopoietic
HMEKT48	Y19567	WO9922243-A1	Cancer
HNGHR74	Y19568	WO9922243-A1	Immune/Hematopoietic
HNHED17	Y19569	WO9922243-A1	Immune/Hematopoietic
HNHEP59	Y19570	WO9922243-A1	Immune/Hematopoietic
HNHFJ25	Y19571	WO9922243-A1	Immune/Hematopoietic
HCPAA69	Y19572	WO9922243-A1	Neural/Sensory
HEAAR07	Y19573	WO9922243-A1	Reproductive
HHGDW43	Y19574	WO9922243-A1	Cancer
HHSDX28	Y19575	WO9922243-A1	Immune/Hematopoietic, Neural/Sensory
HE8ER60	Y19576	WO9922243-A1	Cancer
HMEJQ66	Y19577	WO9922243-A1	Cardiovascular
HRDAD66	Y19578	WO9922243-A1	Cancer
HCMST14	Y19579	WO9922243-A1	Cancer
HCEBA03	Y19580	WO9922243-A1	Neural/Sensory
HJAAM10	Y19582	WO9922243-A1	Cancer
HOHCC74	Y19584	WO9922243-A1	Cancer
HPMFY57	Y19585	WO9922243-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HFXDN63	Y19586	WO9922243-A1	Neural/Sensory
HADCL76	Y19587	WO9922243-A1	Cancer
HMMAS76	Y19588	WO9922243-A1	Endocrine, Immune/Hematopoietic
HMKCG09	Y19589	WO9922243-A1	Digestive, Endocrine, Neural/Sensory
HFPBA88	Y19590	WO9922243-A1	Cancer
HMAH29	Y19596	WO9922243-A1	Cancer
HEMFS60	Y19757	WO9922243-A1	Cancer
HDPVA94	Y25708	WO9938882-A1	Cancer
HDPNE25	Y25709	WO9938882-A1	Cancer
HASCG84	Y25711	WO9938881-A1	Cancer
HDPCY37	Y25712	WO9938881-A1	Cancer
HHEBB10	Y25713	WO9938881-A1	Cancer
HNGJA38	Y25714	WO9938881-A1	Immune/Hematopoietic
HHENL07	Y25715	WO9938881-A1	Immune/Hematopoietic
HKADQ91	Y25716	WO9938881-A1	Cancer
HPMCV18	Y25717	WO9938881-A1	Musculoskeletal, Reproductive



HKGAK22	Y25718	WO9938881-A1	Endocrine, Excretory, Neural/Sensory
HTEHU31	Y25719	WO9938881-A1	Cancer
HFXAM76	Y25720	WO9938881-A1	Cancer
HFXDZ79	Y25721	WO9938881-A1	Neural/Sensory
HOHBC68	Y25722	WO9938881-A1	Cancer
HSVAM81	Y25723	WO9938881-A1	Cancer
HTXDG40	Y25724	WO9938881-A1	Immune/Hematopoietic
HE2FC81	Y25725	WO9938881-A1	Mixed Fetal
HJACE05	Y25726	WO9938881-A1	Cancer
HADCW30	Y25727	WO9938881-A1	Connective/Epithelial
HBMDK25	Y25728	WO9938881-A1	Immune/Hematopoietic
HFXKK25	Y25729	WO9938881-A1	Cancer
HHEMO80	Y25730	WO9938881-A1	Immune/Hematopoietic
HNGEJ53	Y25731	WO9938881-A1	Immune/Hematopoietic
HTBAA70	Y25732	WO9938881-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HSAYB43	Y25734	WO9938881-A1	Immune/Hematopoietic
HSLDS32	Y25735	WO9938881-A1	Cancer
HMIIV27	Y25736	WO9938881-A1	Cancer
HSQEH50	Y25737	WO9938881-A1	Cancer
HKMMU22	Y25738	WO9938881-A1	Excretory
HKMMD13	Y25739	WO9938881-A1	Excretory
HLDNK64	Y25740	WO9938881-A1	Cancer
HRDES01	Y25741	WO9938881-A1	Musculoskeletal
HDTDZ50	Y25742	WO9938881-A1	Cancer
HETAB45	Y25743	WO9938881-A1	Cancer
HFPBD47	Y25744	WO9938881-A1	Cancer
HJMBI18	Y25745	WO9938881-A1	Cancer
HFXHK73	Y25746	WO9938881-A1	Neural/Sensory
HJMBT65	Y25747	WO9938881-A1	Cancer
HWHGZ26	Y25748	WO9938881-A1	Cancer
HADFY83	Y25749	WO9938881-A1	Cancer
HBMTV78	Y25750	WO9938881-A1	Digestive, Immune/Hematopoietic
HTXJM03	Y25751	WO9938881-A1	Cancer
HUSAT94	Y25752	WO9938881-A1	Cancer
HCUEN88	Y25753	WO9938881-A1	Immune/Hematopoietic
HCE3F70	Y25754	WO9938881-A1	Cancer
HCE5F43	Y25755	WO9938881-A1	Cancer
HL2AC08	Y25756	WO9938881-A1	Cancer
HCNSM70	Y25757	WO9938881-A1	Cancer
HDPTQ73	Y25758	WO9938881-A1	Cancer
HTODG13	Y25759	WO9938881-A1	Digestive, Immune/Hematopoietic, Reproductive
HE8DR25	Y25760	WO9938881-A1	Excretory, Mixed Fetal, Neural/Sensory
HSAAO65	Y25761	WO9938881-A1	Cancer
HKGDE09	Y25762	WO9938881-A1	Cancer
HMVBS69	Y25763	WO9938881-A1	Cardiovascular, Immune/Hematopoietic
HSIDU42	Y25764	WO9938881-A1	Cancer

HSKCT36	Y25765	WO9938881-A1	Cancer
HSXBUS9	Y25766	WO9938881-A1	Immune/Hematopoietic, Neural/Sensory
HSSGG82	Y25767	WO9938881-A1	Cancer
HE8CH92	Y25768	WO9938881-A1	Cancer
HYBAR01	Y25769	WO9938881-A1	Musculoskeletal
HTLEF73	Y25770	WO9938881-A1	Cancer
HEOMW84	Y25771	WO9938881-A1	Connective/Epithelial, Immune/Hematopoietic
HKGAR66	Y25772	WO9938881-A1	Cancer
HHPDX20	Y25773	WO9938881-A1	Neural/Sensory
HSICV24	Y25774	WO9938881-A1	Cancer
HCWBE20	Y25775	WO9938881-A1	Immune/Hematopoietic
HSXBM30	Y25776	WO9938881-A1	Cancer
HDPCY37	Y25778	WO9938881-A1	Cancer
HOSFQ65	Y25791	WO9938881-A1	Cancer
HSXBH24	Y25807	WO9938881-A1	Cancer
HUSIG64	Y27567	WO9924836-A1	Cancer
HATCI78	Y27568	WO9924836-A1	Endocrine
HSIDR70	Y27569	WO9924836-A1	Digestive
HFADD53	Y27570	WO9924836-A1	Excretory, Neural/Sensory
HPMGT51	Y27571	WO9924836-A1	Immune/Hematopoietic, Reproductive
HFVAB79	Y27572	WO9924836-A1	Cardiovascular, Digestive, Reproductive
HDTBP51	Y27573	WO9924836-A1	Digestive, Immune/Hematopoietic, Reproductive
HLHFR19	Y27574	WO9924836-A1	Neural/Sensory, Respiratory
HMEET96	Y27575	WO9924836-A1	Cancer
HTXCV12	Y27576	WO9924836-A1	Cancer
HCEFB70	Y27577	WO9924836-A1	Immune/Hematopoietic, Neural/Sensory
HDTAV25	Y27578	WO9924836-A1	Cancer
HSATA21	Y27579	WO9924836-A1	Immune/Hematopoietic
HKIXI03	Y27580	WO9924836-A1	Excretory
HDTDC56	Y27581	WO9924836-A1	Cancer
HLTBF35	Y27582	WO9924836-A1	Cancer
HEPAB80	Y27583	WO9924836-A1	Reproductive
HFOXB13	Y27584	WO9924836-A1	Musculoskeletal
HTOAK16	Y27585	WO9924836-A1	Cardiovascular, Connective/Epithelial, Immune/Hematopoietic
HBXDC63	Y27586	WO9924836-A1	Neural/Sensory
HASAU43	Y27587	WO9924836-A1	Immune/Hematopoietic
HAGEA31	Y27588	WO9924836-A1	Cancer
HTXHB33	Y27590	WO9924836-A1	Immune/Hematopoietic
HMWFT65	Y27591	WO9924836-A1	Immune/Hematopoietic
HNGAZ68	Y27592	WO9924836-A1	Cardiovascular, Immune/Hematopoietic
HTWFH07	Y27593	WO9924836-A1	Immune/Hematopoietic
HMQDF12	Y27594	WO9924836-A1	Cancer
HFABH95	Y27595	WO9924836-A1	Digestive,

			Neural/Sensory, Reproductive
HNGDD48	Y27596	WO9924836-A1	Immune/Hematopoietic
HPMBY46	Y27597	WO9924836-A1	Cancer
HRKPA09	Y27598	WO9924836-A1	Cancer
HAGAQ26	Y27599	WO9924836-A1	Cancer
HCWFL55	Y27600	WO9924836-A1	Immune/Hematopoietic
HKAAE44	Y27601	WO9924836-A1	Cancer
HNGEU90	Y27602	WO9924836-A1	Immune/Hematopoietic
HCFCC07	Y27603	WO9924836-A1	Digestive, Immune/Hematopoietic
HLWBI63	Y27604	WO9924836-A1	Cancer
HDUAC77	Y27605	WO9924836-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HFOYV27	Y27606	WO9924836-A1	Cancer
HGBHI35	Y27607	WO9924836-A1	Cancer
HRDEU27	Y27608	WO9924836-A1	Musculoskeletal
HNGJE50	Y27609	WO9924836-A1	Immune/Hematopoietic
HNH DU48	Y27610	WO9924836-A1	Immune/Hematopoietic
HFXJU68	Y27611	WO9924836-A1	Immune/Hematopoietic, Neural/Sensory
HMMAH60	Y27612	WO9924836-A1	Immune/Hematopoietic
HNGFR31	Y27613	WO9924836-A1	Immune/Hematopoietic
HFPDB26	Y27614	WO9924836-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HFRAW86	Y27615	WO9924836-A1	Neural/Sensory
HTEDX90	Y27616	WO9924836-A1	Reproductive
HTXGG45	Y27617	WO9924836-A1	Immune/Hematopoietic
HTXJI95	Y27618	WO9924836-A1	Immune/Hematopoietic, Reproductive
HLYBD32	Y27619	WO9924836-A1	Immune/Hematopoietic
HROAJ03	Y27621	WO9924836-A1	Cancer
HTXAJ12	Y27622	WO9924836-A1	Immune/Hematopoietic
HKAEL80	Y27623	WO9924836-A1	Connective/Epithelial, Immune/Hematopoietic
HNHFL04	Y27624	WO9924836-A1	Immune/Hematopoietic
HPCAM01	Y27625	WO9924836-A1	Cancer
HJACA79	Y27626	WO9924836-A1	Immune/Hematopoietic
HMSFI26	Y27628	WO9924836-A1	Immune/Hematopoietic
HMSJR08	Y27629	WO9924836-A1	Immune/Hematopoietic
HMWIO93	Y27630	WO9924836-A1	Cancer
HNGAK47	Y27631	WO9924836-A1	Immune/Hematopoietic
HNGAL31	Y27632	WO9924836-A1	Immune/Hematopoietic
HNGIZ06	Y27633	WO9924836-A1	Immune/Hematopoietic
HNHBI75	Y27634	WO9924836-A1	Immune/Hematopoietic
HOFNT24	Y27635	WO9924836-A1	Reproductive
HSAXI95	Y27636	WO9924836-A1	Immune/Hematopoietic
HCMTB45	Y27637	WO9924836-A1	Cardiovascular, Immune/Hematopoietic, Mixed Fetal
HE9CP41	Y27638	WO9924836-A1	Immune/Hematopoietic, Mixed Fetal
HHENV10	Y27639	WO9924836-A1	Immune/Hematopoietic
HSKDD72	Y27640	WO9924836-A1	Digestive,

			Musculoskeletal
HAGDO20	Y27641	WO9924836-A1	Cancer
HCFBH15	Y27642	WO9924836-A1	Immune/Hematopoietic
HSYBX48	Y27643	WO9924836-A1	Cancer
HATDQ62	Y27644	WO9924836-A1	Cancer
HMEJE13	Y27645	WO9924836-A1	Cancer
HNAAF65	Y27646	WO9924836-A1	Cancer
HNFHY30	Y27647	WO9924836-A1	Immune/Hematopoietic
HNFIR81	Y27648	WO9924836-A1	Cancer
HNTBI57	Y27649	WO9924836-A1	Cancer
HSAYR13	Y27650	WO9924836-A1	Immune/Hematopoietic
HTOHV49	Y27651	WO9924836-A1	Immune/Hematopoietic
HSFAG37	Y27652	WO9924836-A1	Cancer
HTXBU52	Y27653	WO9924836-A1	Cancer
HLHFP18	Y27654	WO9924836-A1	Cancer
HFXBW09	Y27655	WO9924836-A1	Neural/Sensory
HNGIO59	Y27656	WO9924836-A1	Immune/Hematopoietic
HNGJF92	Y27657	WO9924836-A1	Immune/Hematopoietic
HMEED18	Y27658	WO9924836-A1	Cancer
HMIAM45	Y27659	WO9924836-A1	Neural/Sensory
HSVVK10	Y27660	WO9924836-A1	Immune/Hematopoietic
HSDHC81	Y27661	WO9924836-A1	Immune/Hematopoietic, Neural/Sensory
HSLCT04	Y27662	WO9924836-A1	Mixed Fetal, Musculoskeletal
HMDAB56	Y27663	WO9924836-A1	Immune/Hematopoietic, Neural/Sensory
HUDBZ89	Y27664	WO9924836-A1	Cancer
HLYCT47	Y27665	WO9924836-A1	Digestive, Immune/Hematopoietic
HOSDJ25	Y27666	WO9924836-A1	Cancer
HADAO89	Y27667	WO9924836-A1	Connective/Epithelial
HMSGB14	Y27668	WO9924836-A1	Cancer
HPMGD01	Y27669	WO9924836-A1	Cancer
HNHFU32	Y27670	WO9924836-A1	Immune/Hematopoietic
HMIAL40	Y27671	WO9924836-A1	Musculoskeletal, Neural/Sensory
HAMFY69	Y27672	WO9924836-A1	Cancer
HBMCT17	Y27673	WO9924836-A1	Immune/Hematopoietic, Neural/Sensory
HEBFI91	Y27674	WO9924836-A1	Neural/Sensory
HHEAH86	Y27675	WO9924836-A1	Cancer
HTPCS72	Y27677	WO9924836-A1	Cancer
HFFAL36	Y27678	WO9924836-A1	Neural/Sensory
HFXBT12	Y27679	WO9924836-A1	Immune/Hematopoietic, Neural/Sensory
HNGJF70	Y27680	WO9924836-A1	Immune/Hematopoietic
HATEE46	Y27681	WO9924836-A1	Cancer
HJMBN89	Y27682	WO9924836-A1	Cancer
HNHEK61	Y27683	WO9924836-A1	Immune/Hematopoietic
HEQAO65	Y27684	WO9924836-A1	Cancer
HFCDV54	Y27685	WO9924836-A1	Cancer
HHEAD14	Y27686	WO9924836-A1	Cancer
HGBHE57	Y27687	WO9924836-A1	Cancer
HGLAF75	Y27688	WO9924836-A1	Digestive, Immune/Hematopoietic,



HPLBM85	Y30814	WO9940100-A1	Cancer
HLMBO76	Y30815	WO9940100-A1	Excretory, Immune/Hematopoietic, Reproductive
HLQDR48	Y30816	WO9940100-A1	Digestive
HOHBY12	Y30817	WO9940100-A1	Musculoskeletal
HOSEK86	Y30818	WO9940100-A1	Cancer
HAJBZ75	Y30819	WO9940100-A1	Cancer
HAGCH75	Y30820	WO9940100-A1	Neural/Sensory
HE8MH91	Y30821	WO9940100-A1	Cancer
HISCJ55	Y30822	WO9940100-A1	Digestive
HKISB57	Y30823	WO9940100-A1	Cancer
HTEBJ71	Y30824	WO9940100-A1	Cancer
HCWGA40	Y30825	WO9940100-A1	Cancer
HFCEW05	Y30826	WO9940100-A1	Cardiovascular, Neural/Sensory
HCEPF19	Y30827	WO9940100-A1	Cancer
HTACZ01	Y30828	WO9940100-A1	Immune/Hematopoietic
HUDAM89	Y30829	WO9940100-A1	Reproductive
HSAXF60	Y30830	WO9940100-A1	Immune/Hematopoietic
HTOGR42	Y30831	WO9940100-A1	Immune/Hematopoietic
HMVBN46	Y30832	WO9940100-A1	Immune/Hematopoietic, Neural/Sensory
HUVEB53	Y30833	WO9940100-A1	Cancer
HSVBU91	Y30834	WO9940100-A1	Cancer
HTXFL30	Y30835	WO9940100-A1	Cancer
HAGAM64	Y30836	WO9940100-A1	Neural/Sensory
HE2PH36	Y30837	WO9940100-A1	Digestive, Immune/Hematopoietic, Mixed Fetal
HGBDY06	Y30838	WO9940100-A1	Cancer
HWBAO62	Y30839	WO9940100-A1	Connective/Epithelial, Immune/Hematopoietic
HBAFJ33	Y30840	WO9940100-A1	Cancer
HFXDJ75	Y30841	WO9940100-A1	Neural/Sensory
HFPCY04	Y30842	WO9940100-A1	Neural/Sensory
HSNMG78	Y30843	WO9940100-A1	Connective/Epithelial, Digestive, Immune/Hematopoietic
HBQAB27	Y30844	WO9940100-A1	Endocrine, Neural/Sensory
HTOJY21	Y30845	WO9940100-A1	Cancer
HHTMM30	Y30846	WO9940100-A1	Cancer
HLTAF58	Y30847	WO9940100-A1	Digestive, Immune/Hematopoietic
HELFG13	Y30848	WO9940100-A1	Cancer
HHFDM48	Y30849	WO9940100-A1	Cardiovascular, Neural/Sensory, Reproductive
HKABI84	Y30850	WO9940100-A1	Cancer
HMVAX72	Y30851	WO9940100-A1	Cancer
HODDN60	Y30852	WO9940100-A1	Cancer
HPMEI44	Y30853	WO9940100-A1	Cancer
HNGJP69	Y30854	WO9940100-A1	Immune/Hematopoietic
HPWBA10	Y30855	WO9940100-A1	Immune/Hematopoietic, Reproductive

HLHCH40	Y30856	WO9940100-A1	Cancer
HTACZ01	Y30857	WO9940100-A1	Immune/Hematopoietic
HTOGR42	Y30858	WO9940100-A1	Immune/Hematopoietic
HTAEK53	Y31811	WO9947538-A1	Cancer
HFCCQ50	Y36224	WO9931117-A1	Cancer
HTLAI54	Y36225	WO9931117-A1	Reproductive
HLWBF94	Y36227	WO9931117-A1	Endocrine, Neural/Sensory, Reproductive
HFKFF78	Y36228	WO9931117-A1	Excretory
HSYBG37	Y36229	WO9931117-A1	Cancer
HTHCA77	Y36230	WO9931117-A1	Immune/Hematopoietic
HNHEZ51	Y36231	WO9931117-A1	Immune/Hematopoietic
HFLAX46	Y36232	WO9931117-A1	Cardiovascular, Musculoskeletal
HFOXO72	Y36233	WO9931117-A1	Cancer
HODDW40	Y36234	WO9931117-A1	Cardiovascular, Immune/Hematopoietic, Reproductive
HSAWG42	Y36235	WO9931117-A1	Immune/Hematopoietic
HBMSK09	Y36236	WO9931117-A1	Digestive, Immune/Hematopoietic, Musculoskeletal
HDDAU16	Y36237	WO9931117-A1	Cancer
HFEBE12	Y36238	WO9931117-A1	Cancer
HFLNB64	Y36239	WO9931117-A1	Cancer
HSAWZ41	Y36240	WO9931117-A1	Immune/Hematopoietic
HNFJF07	Y36241	WO9931117-A1	Immune/Hematopoietic, Neural/Sensory
HNGJO57	Y36242	WO9931117-A1	Immune/Hematopoietic
HE7TM22	Y36243	WO9931117-A1	Mixed Fetal
HFRBR70	Y36244	WO9931117-A1	Cancer
HTHBK35	Y36245	WO9931117-A1	Immune/Hematopoietic
HWABA81	Y36246	WO9931117-A1	Immune/Hematopoietic
HKGAA73	Y36247	WO9931117-A1	Cancer
HKIYP40	Y36248	WO9931117-A1	Cancer
HKMMW74	Y36249	WO9931117-A1	Excretory
HLFBI27	Y36250	WO9931117-A1	Respiratory
HLQCW84	Y36251	WO9931117-A1	Digestive
HBNV22	Y36252	WO9931117-A1	Digestive, Reproductive
HTEAM34	Y36253	WO9931117-A1	Reproductive
HTHDK34	Y36254	WO9931117-A1	Digestive, Immune/Hematopoietic
H6BSG32	Y36255	WO9931117-A1	Cardiovascular, Immune/Hematopoietic, Musculoskeletal
HAECA01	Y36256	WO9931117-A1	Cancer
HDTEL03	Y36257	WO9931117-A1	Cancer
HFXDT43	Y36258	WO9931117-A1	Neural/Sensory
HNGHQ09	Y36259	WO9931117-A1	Immune/Hematopoietic
HHGDF16	Y36260	WO9931117-A1	Cancer
HJBCG12	Y36261	WO9931117-A1	Cancer
HOGAW62	Y36262	WO9931117-A1	Immune/Hematopoietic, Reproductive
HSWBJ74	Y36263	WO9931117-A1	Cancer

HGBHR26	Y36264	WO9931117-A1	Digestive
HKDBF34	Y36265	WO9931117-A1	Cancer
H6EAB28	Y36266	WO9931117-A1	Cancer
HLWAO22	Y36267	WO9931117-A1	Cancer
HAGFH53	Y36268	WO9931117-A1	Cancer
HHENQ22	Y36269	WO9931117-A1	Immune/Hematopoietic
HKMLK53	Y36270	WO9931117-A1	Excretory, Mixed Fetal
HSKGQ58	Y36271	WO9931117-A1	Cancer
HADXB45	Y36272	WO9931117-A1	Cancer
HAIBZ39	Y36273	WO9931117-A1	Cancer
HBXFP23	Y36274	WO9931117-A1	Cancer
HEQBF32	Y36275	WO9931117-A1	Cancer
HETHE81	Y36276	WO9931117-A1	Cancer
HFPAC12	Y36277	WO9931117-A1	Cancer
H6EFA77	Y36278	WO9931117-A1	Cancer
HFXHD88	Y36279	WO9931117-A1	Neural/Sensory
HFOXV65	Y36280	WO9931117-A1	Immune/Hematopoietic, Musculoskeletal, Reproductive
HKADX21	Y36281	WO9931117-A1	Cancer
HPZAB47	Y36282	WO9931117-A1	Cancer
HAGFE79	Y36283	WO9931117-A1	Cancer
HCE1X60	Y36284	WO9931117-A1	Neural/Sensory
HFXKD36	Y36285	WO9931117-A1	Digestive, Musculoskeletal, Neural/Sensory
HBMCU71	Y36286	WO9931117-A1	Immune/Hematopoietic
HTEIV80	Y36287	WO9931117-A1	Reproductive
HFIAP16	Y36288	WO9931117-A1	Musculoskeletal
HODAV86	Y36289	WO9931117-A1	Reproductive
HTEDF80	Y36290	WO9931117-A1	Reproductive
HTODJ69	Y36291	WO9931117-A1	Immune/Hematopoietic
HE6GR02	Y36292	WO9931117-A1	Immune/Hematopoietic, Mixed Fetal
HAPNY86	Y36293	WO9931117-A1	Cancer
HTLDR33	Y36294	WO9931117-A1	Immune/Hematopoietic, Reproductive
HACBI61	Y36295	WO9931117-A1	Cancer
HMEIK34	Y36296	WO9931117-A1	Cancer
HKAAK02	Y36297	WO9931117-A1	Cancer
HEPAA46	Y36298	WO9931117-A1	Reproductive
HFPXC09	Y36299	WO9931117-A1	Mixed Fetal, Neural/Sensory
HLWAA88	Y36300	WO9931117-A1	Cancer
HOHBV89	Y36301	WO9931117-A1	Musculoskeletal, Reproductive
HCEFL57	Y36302	WO9931117-A1	Cancer
HMEKU83	Y36303	WO9931117-A1	Cardiovascular, Immune/Hematopoietic, Reproductive
HOSBY40	Y36304	WO9931117-A1	Digestive, Immune/Hematopoietic, Musculoskeletal
HKFBH93	Y36305	WO9931117-A1	Digestive, Reproductive



HMTAD67	Y36306	WO9931117-A1	Cancer
HTEBP77	Y36307	WO9931117-A1	Immune/Hematopoietic, Reproductive
HE9CO69	Y36308	WO9931117-A1	Cancer
HCACV51	Y36309	WO9931117-A1	Cancer
HHPBI45	Y36310	WO9931117-A1	Cardiovascular, Neural/Sensory
HLQDH79	Y36311	WO9931117-A1	Cancer
HNGFJ67	Y36312	WO9931117-A1	Immune/Hematopoietic
HEIAC52	Y36313	WO9931117-A1	Cancer
HFXXL58	Y36314	WO9931117-A1	Cancer
HMVAM60	Y36315	WO9931117-A1	Cancer
HMVBR22	Y36316	WO9931117-A1	Cancer
HPJCW04	Y36317	WO9931117-A1	Reproductive
HSIDJ81	Y36318	WO9931117-A1	Digestive
HSLFU05	Y36319	WO9931117-A1	Cancer
HEQAK71	Y36320	WO9931117-A1	Cancer
HOSEQ49	Y36321	WO9931117-A1	Cancer
HRAAM50	Y36322	WO9931117-A1	Excretory, Immune/Hematopoietic, Mixed Fetal
HSDFW45	Y36323	WO9931117-A1	Neural/Sensory
HSLCQ82	Y36324	WO9931117-A1	Cancer
HSSFT08	Y36325	WO9931117-A1	Musculoskeletal
HTOIW31	Y36326	WO9931117-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HTXKQ85	Y36327	WO9931117-A1	Immune/Hematopoietic, Musculoskeletal, Reproductive
HUFBK08	Y36328	WO9931117-A1	Digestive, Musculoskeletal
HBJEE48	Y36330	WO9931117-A1	Cancer
HBXGH74	Y36331	WO9931117-A1	Neural/Sensory
HISBM03	Y36332	WO9931117-A1	Cancer
HETCH46	Y36333	WO9931117-A1	Cancer
HFPCX09	Y36335	WO9931117-A1	Mixed Fetal, Neural/Sensory
HLWAA88	Y36336	WO9931117-A1	Cancer
HCEFL57	Y36337	WO9931117-A1	Cancer
HETHE81	Y36650	WO9931117-A1	Cancer
HTGAU75	Y38386	WO9935158-A1	Immune/Hematopoietic
HTTDP47	Y38387	WO9935158-A1	Cancer
HTXJQ11	Y38388	WO9935158-A1	Cancer
HADCO45	Y38389	WO9935158-A1	Cancer
HMIAL37	Y38390	WO9935158-A1	Cancer
HNGDU40	Y38391	WO9935158-A1	Immune/Hematopoietic
HFXBO84	Y38392	WO9935158-A1	Neural/Sensory
HLLAX19	Y38393	WO9935158-A1	Cancer
HPMAG94	Y38394	WO9935158-A1	Cancer
HSVAK93	Y38395	WO9935158-A1	Cancer
HMQBO88	Y38396	WO9935158-A1	Cancer
HMQBU45	Y38397	WO9935158-A1	Immune/Hematopoietic
HMWAJ53	Y38398	WO9935158-A1	Immune/Hematopoietic
HCUGO12	Y38401	WO9935158-A1	Digestive, Immune/Hematopoietic,

			Mixed Fetal
HPFCX44	Y38402	WO9935158-A1	Cancer
HCUBV79	Y38403	WO9935158-A1	Immune/Hematopoietic, Neural/Sensory
HLQBV04	Y38404	WO9935158-A1	Cancer
HMADW66	Y38405	WO9935158-A1	Cancer
HLDBE54	Y38406	WO9935158-A1	Digestive, Reproductive
HFTAB66	Y38407	WO9935158-A1	Digestive, Neural/Sensory
HEOMQ63	Y38408	WO9935158-A1	Digestive, Immune/Hematopoietic
HDPJM30	Y38409	WO9935158-A1	Immune/Hematopoietic, Neural/Sensory
HCFMG62	Y38410	WO9935158-A1	Cancer
HJMAG88	Y38411	WO9935158-A1	Cancer
HKAAH36	Y38412	WO9935158-A1	Connective/Epithelial, Reproductive
HMADS41	Y38413	WO9935158-A1	Cancer
HMEFT85	Y38414	WO9935158-A1	Cancer
HMSBX80	Y38415	WO9935158-A1	Immune/Hematopoietic, Reproductive
HNGCL23	Y38416	WO9935158-A1	Immune/Hematopoietic
HPIBO15	Y38418	WO9935158-A1	Cancer
HCYBG92	Y38419	WO9935158-A1	Cancer
HMDAQ29	Y38420	WO9935158-A1	Neural/Sensory, Reproductive
HSYBI49	Y38421	WO9935158-A1	Cancer
HDTAB58	Y38422	WO9935158-A1	Cancer
HFTAB66	Y38423	WO9935158-A1	Digestive, Neural/Sensory
HDPBX23	Y38424	WO9935158-A1	Immune/Hematopoietic, Neural/Sensory
HCFMG62	Y38425	WO9935158-A1	Cancer
HKAAH36	Y38426	WO9935158-A1	Connective/Epithelial, Reproductive
HKAAH36	Y38427	WO9935158-A1	Connective/Epithelial, Reproductive
HMADS41	Y38428	WO9935158-A1	Cancer
HNTBI26	Y38429	WO9935158-A1	Cancer
HCYBI36	Y38430	WO9935158-A1	Cancer
HTHBJ48	Y41161	US5981231-A	Digestive, Immune/Hematopoietic
HDQAC88	Y41164	US5981230-A	Cancer
HKGCR51	Y41308	WO9947540-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HPMDK28	Y41309	WO9947540-A1	Cancer
HLDCD04	Y41310	WO9947540-A1	Cancer
HLDON23	Y41311	WO9947540-A1	Cancer
HLDRM43	Y41312	WO9947540-A1	Digestive, Reproductive
HLQAM28	Y41313	WO9947540-A1	Digestive, Reproductive
HLTDE74	Y41314	WO9947540-A1	Cancer
HLTFA64	Y41315	WO9947540-A1	Cancer

HMCIFY13	Y41316	WO9947540-A1	Immune/Hematopoietic
HMMBD35	Y41317	WO9947540-A1	Cancer
HMQCY03	Y41318	WO9947540-A1	Digestive, Immune/Hematopoietic
HMSBX84	Y41319	WO9947540-A1	Immune/Hematopoietic
HMSKI86	Y41320	WO9947540-A1	Cancer
HMVBS81	Y41321	WO9947540-A1	Cancer
HMWEB02	Y41322	WO9947540-A1	Cancer
HMZAD77	Y41323	WO9947540-A1	Cancer
HNFIY77	Y41324	WO9947540-A1	Cancer
HNHEK85	Y41325	WO9947540-A1	Immune/Hematopoietic, Mixed Fetal
HNHEU93	Y41326	WO9947540-A1	Immune/Hematopoietic
HODAH74	Y41327	WO9947540-A1	Connective/Epithelial, Reproductive, Respiratory
HODCU34	Y41328	WO9947540-A1	Cancer
HODCZ09	Y41329	WO9947540-A1	Reproductive
HISCF16	Y41330	WO9947540-A1	Cancer
HOGAG15	Y41331	WO9947540-A1	Cancer
HPIBO48	Y41332	WO9947540-A1	Cancer
HPMFP40	Y41333	WO9947540-A1	Reproductive
HPRCU95	Y41334	WO9947540-A1	Musculoskeletal, Reproductive
HPTTG19	Y41335	WO9947540-A1	Endocrine, Immune/Hematopoietic
HRDDV47	Y41337	WO9947540-A1	Cancer
HRDEN56	Y41338	WO9947540-A1	Musculoskeletal
HSFAN12	Y41339	WO9947540-A1	Cardiovascular
HSQCM10	Y41340	WO9947540-A1	Cancer
HSVAT68	Y41341	WO9947540-A1	Excretory, Reproductive
HSXEC75	Y41342	WO9947540-A1	Cancer
HTDAI54	Y41343	WO9947540-A1	Cancer
HTEIT45	Y41344	WO9947540-A1	Reproductive
HTGBE48	Y41345	WO9947540-A1	Immune/Hematopoietic, Reproductive
HTLEP53	Y41346	WO9947540-A1	Neural/Sensory, Reproductive
HTTBI76	Y41347	WO9947540-A1	Cancer
HTWKG71	Y41348	WO9947540-A1	Immune/Hematopoietic
HTXDN32	Y41349	WO9947540-A1	Cancer
HTSGX80	Y41350	WO9947540-A1	Cancer
HTXEY51	Y41351	WO9947540-A1	Endocrine, Immune/Hematopoietic, Mixed Fetal
HTXFH55	Y41352	WO9947540-A1	Cardiovascular, Immune/Hematopoietic
HTXJW17	Y41353	WO9947540-A1	Digestive, Immune/Hematopoietic
HUFCJ30	Y41354	WO9947540-A1	Cancer
HWAAP70	Y41355	WO9947540-A1	Immune/Hematopoietic
HWABW49	Y41356	WO9947540-A1	Immune/Hematopoietic
HWBDP28	Y41357	WO9947540-A1	Cancer
HWDAC39	Y41358	WO9947540-A1	Connective/Epithelial
HWHGQ49	Y41359	WO9947540-A1	Cancer

HJPAD75	Y41360	WO9947540-A1	Cancer
HLDRP33	Y41361	WO9947540-A1	Digestive, Neural/Sensory
HMSIE02	Y41362	WO9947540-A1	Cancer
HNGFE55	Y41363	WO9947540-A1	Immune/Hematopoietic
HRAAJ19	Y41365	WO9947540-A1	Cancer
HSAWV96	Y41366	WO9947540-A1	Immune/Hematopoietic, Neural/Sensory
HSBBT37	Y41367	WO9947540-A1	Cancer
HSDZR57	Y41368	WO9947540-A1	Cancer
HCECQ07	Y41369	WO9947540-A1	Cancer
HWBCP79	Y41370	WO9947540-A1	Immune/Hematopoietic, Reproductive
HYAAL70	Y41371	WO9947540-A1	Cancer
HYAAY86	Y41372	WO9947540-A1	Immune/Hematopoietic
HAPBS03	Y41373	WO9947540-A1	Cancer
HBJLC01	Y41374	WO9947540-A1	Immune/Hematopoietic
HBLKD56	Y41375	WO9947540-A1	Musculoskeletal
HCENK38	Y41376	WO9947540-A1	Cancer
HE6GA29	Y41379	WO9947540-A1	Mixed Fetal
HETHO95	Y41381	WO9947540-A1	Digestive, Reproductive
HFCFJ18	Y41382	WO9947540-A1	Cancer
HFPBM30	Y41383	WO9947540-A1	Neural/Sensory
HFXKT05	Y41384	WO9947540-A1	Cancer
HKB1E57	Y41385	WO9947540-A1	Cancer
HLWAD77	Y41386	WO9947540-A1	Cancer
HLWAY54	Y41387	WO9947540-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HNGBU28	Y41388	WO9947540-A1	Immune/Hematopoietic
HOUHH51	Y41389	WO9947540-A1	Cancer
HRAAB15	Y41390	WO9947540-A1	Digestive, Excretory
HSAVH65	Y41391	WO9947540-A1	Digestive, Immune/Hematopoietic, Reproductive
HSDGN55	Y41392	WO9947540-A1	Cancer
HSXAH81	Y41393	WO9947540-A1	Cancer
HSXBX80	Y41394	WO9947540-A1	Cancer
HTEHV08	Y41395	WO9947540-A1	Cancer
HUFAK67	Y41396	WO9947540-A1	Digestive, Immune/Hematopoietic, Reproductive
HUSXS50	Y41397	WO9947540-A1	Cancer
HAPON17	Y41398	WO9947540-A1	Cancer
HATAC53	Y41399	WO9947540-A1	Cancer
HAMFK58	Y41400	WO9947540-A1	Cancer
HLYCH68	Y41401	WO9947540-A1	Cancer
HCUHK65	Y41402	WO9947540-A1	Cancer
HLDCD04	Y41403	WO9947540-A1	Cancer
HOUHH51	Y41404	WO9947540-A1	Cancer
HSLCQ82	Y41571	WO9947540-A1	Cancer
HCGMD59	Y45257	WO9946289-A1	Cancer
HCNSD76	Y45258	WO9946289-A1	Digestive
HCNSD93	Y45259	WO9946289-A1	Digestive

HCWBE22	Y45260	WO9946289-A1	Immune/Hematopoietic, Neural/Sensory
HFEAN33	Y45261	WO9946289-A1	Cancer
HCWUM50	Y45262	WO9946289-A1	Cancer
HDHIA94	Y45263	WO9946289-A1	Excretory, Neural/Sensory
HDPAE76	Y45264	WO9946289-A1	Cancer
HDPIO54	Y45265	WO9946289-A1	Immune/Hematopoietic, Reproductive
HDPNC61	Y45266	WO9946289-A1	Cancer
HDPND46	Y45267	WO9946289-A1	Immune/Hematopoietic
HDPSU13	Y45268	WO9946289-A1	Immune/Hematopoietic
HDTGC73	Y45269	WO9946289-A1	Cancer
HE2PD49	Y45270	WO9946289-A1	Cancer
HEEAJ02	Y45271	WO9946289-A1	Cancer
HELHD64	Y45272	WO9946289-A1	Cancer
HEPAD91	Y45273	WO9946289-A1	Digestive, Reproductive
HEQBH65	Y45274	WO9946289-A1	Immune/Hematopoietic, Reproductive
HETCO02	Y45275	WO9946289-A1	Cancer
HFAUO78	Y45276	WO9946289-A1	Cancer
HFKEE48	Y45277	WO9946289-A1	Cancer
HFKFG02	Y45278	WO9946289-A1	Excretory, Immune/Hematopoietic, Neural/Sensory
H2CBN14	Y45279	WO9946289-A1	Cancer
HHFFJ48	Y45280	WO9946289-A1	Cardiovascular, Immune/Hematopoietic
HILCF66	Y45281	WO9946289-A1	Cancer
HKABN45	Y45282	WO9946289-A1	Cancer
HKDBK22	Y45284	WO9946289-A1	Excretory
HKGAZ06	Y45286	WO9946289-A1	Immune/Hematopoietic
HKGCK61	Y45287	WO9946289-A1	Cancer
HFEAN33	Y45288	WO9946289-A1	Cancer
HDHIA94	Y45289	WO9946289-A1	Excretory, Neural/Sensory
HDPJO39	Y52479	WO9940184-A1	Cancer
HNTCF82	Y58185	US6004780-A	Cardiovascular, Connective/Epithelial, Reproductive
HETAB62	Y59285	WO200004183-A1	Cancer
HSYAE36	Y59286	WO200004183-A1	Cancer
HKAPI15	Y68800	WO200005371-A1	Connective/Epithelial
HUJCT9C	Y72090	WO200068247-A2	Cancer
HMGBM65	Y72091	WO200068247-A2	Cancer
HATEE38	Y72092	WO200068247-A2	Cancer
HCHAK72	Y72093	WO200068247-A2	Cancer
HHFBJ67	Y72094	WO200068247-A2	Cardiovascular, Neural/Sensory
HTTJK5C	Y72095	WO200068247-A2	Cancer
HWLGJ11	Y72096	WO200068247-A2	Digestive
HTLEG15	Y72097	WO200068247-A2	Cancer
HAGAS16	Y72098	WO200068247-A2	Neural/Sensory
HATEE38	Y72108	WO200068247-A2	Cancer
HKABZ65	Y76124	WO9958660-A1	Connective/Epithelial

HNGIC80	Y76125	WO9958660-A1	Immune/Hematopoietic
HDPUG50	Y76126	WO9958660-A1	Cancer
HAEAB66	Y76127	WO9958660-A1	Cancer
HHEPF59	Y76128	WO9958660-A1	Cancer
HE9BK23	Y76129	WO9958660-A1	Digestive, Mixed Fetal
HCYBI36	Y76130	WO9958660-A1	Cancer
HSSDX51	Y76131	WO9958660-A1	Cancer
HSDAJ46	Y76132	WO9958660-A1	Cancer
HRACG45	Y76133	WO9958660-A1	Cancer
HAPPW30	Y76134	WO9958660-A1	Cancer
HE2ES51	Y76135	WO9958660-A1	Cancer
HTXDW56	Y76136	WO9958660-A1	Cancer
HDPKI93	Y76138	WO9958660-A1	Cancer
HDLAC10	Y76139	WO9958660-A1	Cancer
HDPOH06	Y76140	WO9958660-A1	Cancer
HCE4G61	Y76141	WO9958660-A1	Cancer
HCWUI13	Y76142	WO9958660-A1	Immune/Hematopoietic
HDPSP01	Y76143	WO9958660-A1	Cancer
HHPEN62	Y76144	WO9958660-A1	Cancer
HUKBT29	Y76145	WO9958660-A1	Cancer
HARAP48	Y76146	WO9958660-A1	Cancer
HBIMB51	Y76147	WO9958660-A1	Connective/Epithelial, Reproductive
HE8DX88	Y76148	WO9958660-A1	Mixed Fetal
HNGHT03	Y76149	WO9958660-A1	Immune/Hematopoietic
HWABU17	Y76150	WO9958660-A1	Cancer
HCE5F84	Y76151	WO9958660-A1	Cancer
HBXCD55	Y76152	WO9958660-A1	Cancer
HOVCB25	Y76153	WO9958660-A1	Reproductive
HSYAV66	Y76154	WO9958660-A1	Digestive, Immune/Hematopoietic
HFPCT29	Y76155	WO9958660-A1	Neural/Sensory
HAWAT25	Y76156	WO9958660-A1	Cancer
HNHFR04	Y76157	WO9958660-A1	Immune/Hematopoietic
HOSFT61	Y76158	WO9958660-A1	Cancer
HBJIO81	Y76159	WO9958660-A1	Immune/Hematopoietic
HADCL55	Y76160	WO9958660-A1	Cancer
HAGGJ80	Y76161	WO9958660-A1	Cancer
HAIBO81	Y76162	WO9958660-A1	Neural/Sensory
HBBBC37	Y76163	WO9958660-A1	Cancer
HBJMX85	Y76164	WO9958660-A1	Cancer
HCEES66	Y76165	WO9958660-A1	Digestive, Neural/Sensory
HCEMP62	Y76166	WO9958660-A1	Cancer
HE2FB90	Y76167	WO9958660-A1	Cancer
HE9DS56	Y76168	WO9958660-A1	Cancer
HTOHJ89	Y76169	WO9958660-A1	Immune/Hematopoietic
HASCE69	Y76171	WO9958660-A1	Cancer
HHTLH52	Y76172	WO9958660-A1	Neural/Sensory, Reproductive
HOUCT90	Y76174	WO9958660-A1	Connective/Epithelial
HCFLR78	Y76175	WO9958660-A1	Cancer
HTOHT18	Y76176	WO9958660-A1	Cancer
HKPMB11	Y76177	WO9958660-A1	Digestive, Excretory,

			Musculoskeletal
HNHFS38	Y76178	WO9958660-A1	Cancer
HAIBU10	Y76179	WO9958660-A1	Cancer
HAPOK30	Y76180	WO9958660-A1	Cancer
HCWUA22	Y76182	WO9958660-A1	Immune/Hematopoietic
HDSAG91	Y76183	WO9958660-A1	Immune/Hematopoietic
HNEDJ35	Y76184	WO9958660-A1	Immune/Hematopoietic, Reproductive
HTHBH29	Y76185	WO9958660-A1	Immune/Hematopoietic, Mixed Fetal, Reproductive
H7TBA62	Y76186	WO9958660-A1	Cancer
HNGIO50	Y76187	WO9958660-A1	Immune/Hematopoietic
HMLAW81	Y76188	WO9958660-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HMMCJ60	Y76189	WO9958660-A1	Immune/Hematopoietic, Musculoskeletal
HDPIO09	Y76190	WO9958660-A1	Cancer
HHFHH34	Y76191	WO9958660-A1	Cardiovascular
HISCL83	Y76192	WO9958660-A1	Digestive
HTOAI70	Y76193	WO9958660-A1	Immune/Hematopoietic
HSDER95	Y76194	WO9958660-A1	Digestive, Neural/Sensory
HNECL25	Y76195	WO9958660-A1	Immune/Hematopoietic
HNFGZ45	Y76196	WO9958660-A1	Cardiovascular, Digestive, Immune/Hematopoietic
HHGCU49	Y76197	WO9958660-A1	Cancer
HETDT81	Y76199	WO9958660-A1	Digestive, Immune/Hematopoietic, Reproductive
HHLBA14	Y76200	WO9958660-A1	Cancer
HLTBU43	Y76201	WO9958660-A1	Immune/Hematopoietic
HNTSJ84	Y76202	WO9958660-A1	Cancer
HOHCG16	Y76203	WO9958660-A1	Digestive, Musculoskeletal
HTHCB31	Y76204	WO9958660-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HUKAM16	Y76205	WO9958660-A1	Cancer
HLDOJ66	Y76206	WO9958660-A1	Digestive
HTXKF10	Y76207	WO9958660-A1	Immune/Hematopoietic
HPMAI22	Y76208	WO9958660-A1	Reproductive
HL2AG57	Y76209	WO9958660-A1	Cancer
HUSAM59	Y76210	WO9958660-A1	Cancer
HNGGR26	Y76211	WO9958660-A1	Immune/Hematopoietic
HTLCX30	Y76212	WO9958660-A1	Reproductive
HCEBC87	Y76213	WO9958660-A1	Cancer
HATCB92	Y76214	WO9958660-A1	Endocrine
HLHAL68	Y76216	WO9958660-A1	Respiratory
HEOMR73	Y76217	WO9958660-A1	Immune/Hematopoietic
HETIB83	Y76218	WO9958660-A1	Cancer
HJPDD28	Y76219	WO9958660-A1	Cancer
HBAMB15	Y76220	WO9958660-A1	Cardiovascular, Excretory,

			Reproductive
HBAFQ33	Y76221	WO9958660-A1	Cancer
HTOAI70	Y76222	WO9958660-A1	Immune/Hematopoietic
HJPDD28	Y76223	WO9958660-A1	Cancer
HRACG45	Y76266	WO9958660-A1	Cancer
HBXCD55	Y76303	WO9958660-A1	Cancer
HOSFT61	Y76325	WO9958660-A1	Cancer
HWBBP10	Y86215	WO9966041-A1	Immune/Hematopoietic, Neural/Sensory
HWBDO80	Y86216	WO9966041-A1	Immune/Hematopoietic, Musculoskeletal, Reproductive
HWHGU54	Y86217	WO9966041-A1	Connective/Epithelial
HYACI76	Y86218	WO9966041-A1	Cancer
HBHMA23	Y86219	WO9966041-A1	Cancer
HCE3G20	Y86220	WO9966041-A1	Cancer
HCEJP80	Y86221	WO9966041-A1	Cardiovascular, Neural/Sensory
HCUDD24	Y86222	WO9966041-A1	Digestive, Immune/Hematopoietic, Reproductive
HDPTD15	Y86223	WO9966041-A1	Immune/Hematopoietic
HDPWU34	Y86224	WO9966041-A1	Cancer
HEOOV79	Y86225	WO9966041-A1	Cancer
HFKET93	Y86226	WO9966041-A1	Excretory, Immune/Hematopoietic, Neural/Sensory
HFTDL56	Y86227	WO9966041-A1	Cancer
HFXJX44	Y86228	WO9966041-A1	Cancer
HKACU58	Y86229	WO9966041-A1	Cancer
HKFBC53	Y86230	WO9966041-A1	Cancer
HLTHR66	Y86231	WO9966041-A1	Cancer
HLYBA69	Y86232	WO9966041-A1	Cancer
HNTMX29	Y86233	WO9966041-A1	Cancer
HNTNC20	Y86234	WO9966041-A1	Cancer
HNTNI01	Y86235	WO9966041-A1	Cancer
HPIBW65	Y86236	WO9966041-A1	Cancer
HSMBE69	Y86237	WO9966041-A1	Cancer
HT4FW61	Y86238	WO9966041-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HYABK95	Y86239	WO9966041-A1	Cancer
HYACE88	Y86240	WO9966041-A1	Cancer
HOABR60	Y86241	WO9966041-A1	Cancer
HAPOM45	Y86243	WO9966041-A1	Cardiovascular, Digestive
HCEJQ69	Y86244	WO9966041-A1	Cancer
HAGFI62	Y86245	WO9966041-A1	Cancer
HAGGS43	Y86246	WO9966041-A1	Neural/Sensory
HBJHP03	Y86247	WO9966041-A1	Immune/Hematopoietic, Reproductive
HCHPF68	Y86248	WO9966041-A1	Reproductive
HDPJF37	Y86249	WO9966041-A1	Cancer
HSDEZ20	Y86250	WO9966041-A1	Neural/Sensory
HTEKU58	Y86251	WO9966041-A1	Cancer
HLTBL58	Y86252	WO9966041-A1	Immune/Hematopoietic,



			Musculoskeletal, Neural/Sensory
HPWDJ42	Y86253	WO9966041-A1	Digestive, Reproductive
HRACD15	Y86254	WO9966041-A1	Cancer
HSIAC80	Y86255	WO9966041-A1	Cancer
HAGFD18	Y86256	WO9966041-A1	Cancer
HAJAP76	Y86257	WO9966041-A1	Cancer
HDTGC86	Y86258	WO9966041-A1	Digestive, Immune/Hematopoietic, Reproductive
HAGDI35	Y86259	WO9966041-A1	Cancer
HELHN47	Y86260	WO9966041-A1	Cancer
HPRBC80	Y86261	WO9966041-A1	Cancer
HAQAR23	Y86262	WO9966041-A1	Cancer
HAIFL18	Y86263	WO9966041-A1	Digestive, Immune/Hematopoietic
HJPAY76	Y86264	WO9966041-A1	Cancer
HUSXE77	Y86265	WO9966041-A1	Cancer
HUFEF62	Y86266	WO9966041-A1	Digestive
HTWJK32	Y86267	WO9966041-A1	Cancer
HTWDF76	Y86268	WO9966041-A1	Immune/Hematopoietic
HTPBN68	Y86269	WO9966041-A1	Digestive
HTOIY21	Y86270	WO9966041-A1	Immune/Hematopoietic
HTLDD53	Y86271	WO9966041-A1	Connective/Epithelial, Digestive, Reproductive
HTLFG05	Y86272	WO9966041-A1	Cancer
HDPXR23	Y86273	WO9966041-A1	Digestive, Immune/Hematopoietic
HSIAC45	Y86274	WO9966041-A1	Digestive, Immune/Hematopoietic
HSRGW16	Y86275	WO9966041-A1	Cancer
HSSJC35	Y86276	WO9966041-A1	Cancer
HTEAX23	Y86277	WO9966041-A1	Reproductive
HTGCH22	Y86278	WO9966041-A1	Immune/Hematopoietic, Mixed Fetal, Reproductive
HTJMA95	Y86279	WO9966041-A1	Cancer
HHEAA08	Y86280	WO9966041-A1	Immune/Hematopoietic
HBQAA49	Y86281	WO9966041-A1	Neural/Sensory
HDPBI32	Y86282	WO9966041-A1	Excretory, Immune/Hematopoietic, Neural/Sensory
HBIBF16	Y86283	WO9966041-A1	Neural/Sensory
HBCAY05	Y86284	WO9966041-A1	Cancer
HCUCK44	Y86285	WO9966041-A1	Cancer
HCE2W56	Y86286	WO9966041-A1	Cancer
HCWAG01	Y86287	WO9966041-A1	Immune/Hematopoietic
HDRMI82	Y86289	WO9966041-A1	Cancer
HEPCU48	Y86290	WO9966041-A1	Cancer
H DPRK33	Y86291	WO9966041-A1	Immune/Hematopoietic, Mixed Fetal
HKGAX42	Y86292	WO9966041-A1	Digestive, Immune/Hematopoietic, Reproductive

HLMAZ95	Y86293	WO9966041-A1	Cancer
HLMFC07	Y86294	WO9966041-A1	Digestive, Immune/Hematopoietic
HL2AG87	Y86295	WO9966041-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HKGCO27	Y86296	WO9966041-A1	Cancer
HLDCE79	Y86297	WO9966041-A1	Digestive
HERAD40	Y86298	WO9966041-A1	Connective/Epithelial
HFOXB55	Y86299	WO9966041-A1	Cancer
HFVGZ42	Y86300	WO9966041-A1	Cancer
HNHAF39	Y86301	WO9966041-A1	Immune/Hematopoietic
HNTSW57	Y86302	WO9966041-A1	Cancer
HOGCK20	Y86303	WO9966041-A1	Cancer
HLYES38	Y86305	WO9966041-A1	Immune/Hematopoietic, Reproductive
HMECK83	Y86306	WO9966041-A1	Cardiovascular
HMQAG66	Y86308	WO9966041-A1	Immune/Hematopoietic
HWBBP10	Y86309	WO9966041-A1	Immune/Hematopoietic, Neural/Sensory
HAPAK52	Y86310	WO9966041-A1	Cancer
HDPWU34	Y86311	WO9966041-A1	Cancer
HKACU58	Y86312	WO9966041-A1	Cancer
HLDBQ19	Y86314	WO9966041-A1	Cancer
HNTMX29	Y86315	WO9966041-A1	Cancer
HOABR60	Y86316	WO9966041-A1	Cancer
HPWDJ42	Y86317	WO9966041-A1	Digestive, Reproductive
HPWDJ42	Y86318	WO9966041-A1	Digestive, Reproductive
HRACD15	Y86319	WO9966041-A1	Cancer
HPRBC80	Y86320	WO9966041-A1	Cancer
HUFEF62	Y86321	WO9966041-A1	Digestive
HTLFG05	Y86322	WO9966041-A1	Cancer
HDPXR23	Y86323	WO9966041-A1	Digestive, Immune/Hematopoietic
HSRGW16	Y86324	WO9966041-A1	Cancer
HDPBI32	Y86327	WO9966041-A1	Excretory, Immune/Hematopoietic, Neural/Sensory
HDRMI82	Y86328	WO9966041-A1	Cancer
HKGCO27	Y86330	WO9966041-A1	Cancer
HNTSW57	Y86332	WO9966041-A1	Cancer
HOGCK20	Y86333	WO9966041-A1	Cancer
HNTMX29	Y86388	WO9966041-A1	Cancer
HPRBC80	Y86463	WO9966041-A1	Cancer
HTLFG05	Y86488	WO9966041-A1	Cancer
HDPXR23	Y86489	WO9966041-A1	Digestive, Immune/Hematopoietic
HSRGW16	Y86496	WO9966041-A1	Cancer
HDRMI82	Y86532	WO9966041-A1	Cancer
HNTSW57	Y86571	WO9966041-A1	Cancer
HISCN02	Y87064	WO200004140-A1	Digestive
HHGDM70	Y87065	WO200004140-A1	Immune/Hematopoietic
HHPGO40	Y87066	WO200004140-A1	Cancer
HAMGG68	Y87067	WO200004140-A1	Cancer

HAPOM49	Y87068	WO200004140-A1	Cancer
HBGBA69	Y87069	WO200004140-A1	Cancer
HBJFJ26	Y87070	WO200004140-A1	Cancer
HCEDH38	Y87071	WO200004140-A1	Mixed Fetal, Neural/Sensory
HDPOJ08	Y87072	WO200004140-A1	Cancer
HDPRX82	Y87073	WO200004140-A1	Cancer
HELK31	Y87074	WO200004140-A1	Cancer
HFPCX64	Y87075	WO200004140-A1	Mixed Fetal, Neural/Sensory
HFXDO60	Y87076	WO200004140-A1	Neural/Sensory
HAUAI83	Y87077	WO200004140-A1	Reproductive
HKGAH42	Y87078	WO200004140-A1	Neural/Sensory
HMIAP86	Y87079	WO200004140-A1	Cancer
HMUAP70	Y87080	WO200004140-A1	Cancer
HRACJ35	Y87081	WO200004140-A1	Cancer
HTWDE26	Y87082	WO200004140-A1	Cancer
HBGBB44	Y87083	WO200004140-A1	Cancer
HBAFA02	Y87084	WO200004140-A1	Cancer
H2CBT75	Y87085	WO200004140-A1	Cancer
HAGDQ42	Y87086	WO200004140-A1	Cancer
HBMCI42	Y87087	WO200004140-A1	Immune/Hematopoietic, Reproductive
HLCDA16	Y87089	WO200004140-A1	Cancer
HELHL48	Y87090	WO200004140-A1	Cancer
HISAQ04	Y87091	WO200004140-A1	Digestive, Neural/Sensory, Reproductive
HJACB89	Y87092	WO200004140-A1	Cancer
HTECC05	Y87093	WO200004140-A1	Cancer
HBJLF01	Y87094	WO200004140-A1	Cancer
HBXGP60	Y87095	WO200004140-A1	Cancer
HCE5B20	Y87096	WO200004140-A1	Mixed Fetal, Neural/Sensory
HCMSQ56	Y87097	WO200004140-A1	Cancer
HCNAH57	Y87098	WO200004140-A1	Digestive
HCUEP91	Y87099	WO200004140-A1	Immune/Hematopoietic
HDPCJ91	Y87100	WO200004140-A1	Cancer
HDPGK25	Y87101	WO200004140-A1	Cancer
HE2DY70	Y87102	WO200004140-A1	Immune/Hematopoietic, Mixed Fetal, Musculoskeletal
HE2NV57	Y87103	WO200004140-A1	Cancer
HETBR16	Y87104	WO200004140-A1	Digestive, Immune/Hematopoietic, Reproductive
HFXDG13	Y87105	WO200004140-A1	Cancer
HFXKY27	Y87106	WO200004140-A1	Neural/Sensory
HHPEC09	Y87107	WO200004140-A1	Cancer
HISAD54	Y87108	WO200004140-A1	Cancer
HJBCY35	Y87109	WO200004140-A1	Cancer
HKAEA19	Y87110	WO200004140-A1	Cancer
HKGDL36	Y87111	WO200004140-A1	Cancer
HLDBS43	Y87112	WO200004140-A1	Cancer
HLWAD92	Y87113	WO200004140-A1	Cancer
HLYBI15	Y87114	WO200004140-A1	Immune/Hematopoietic

HMEJE05	Y87115	WO200004140-A1	Cancer
HNGIX55	Y87116	WO200004140-A1	Immune/Hematopoietic
HNHEX30	Y87117	WO200004140-A1	Immune/Hematopoietic
HPJBI33	Y87118	WO200004140-A1	Reproductive
HRABA80	Y87119	WO200004140-A1	Excretory
HRACD80	Y87120	WO200004140-A1	Excretory, Reproductive
HSLCX03	Y87121	WO200004140-A1	Cancer
HT5GJ57	Y87122	WO200004140-A1	Cancer
HTACS42	Y87123	WO200004140-A1	Cancer
HTEKE40	Y87124	WO200004140-A1	Cancer
HTOBX69	Y87125	WO200004140-A1	Cancer
HUVEO77	Y87126	WO200004140-A1	Reproductive
H2CBG48	Y87127	WO200004140-A1	Cancer
H2CBU83	Y87128	WO200004140-A1	Cancer
HAPNY94	Y87129	WO200004140-A1	Cancer
HBJHZ58	Y87130	WO200004140-A1	Immune/Hematopoietic, Reproductive
HCE2B33	Y87131	WO200004140-A1	Cancer
HDPBQ02	Y87132	WO200004140-A1	Immune/Hematopoietic
HFIYI70	Y87133	WO200004140-A1	Cancer
HDPOZ56	Y87134	WO200004140-A1	Cancer
HAPOM49	Y87136	WO200004140-A1	Cancer
HBJFJ26	Y87137	WO200004140-A1	Cancer
HCNUA40	Y87138	WO200004140-A1	Cancer
HCEBW71	Y87139	WO200004140-A1	Mixed Fetal, Neural/Sensory
HCEBW71	Y87140	WO200004140-A1	Mixed Fetal, Neural/Sensory
HAUAI83	Y87141	WO200004140-A1	Reproductive
HFLQB16	Y87143	WO200004140-A1	Cancer
HAGFY16	Y87144	WO200004140-A1	Cancer
HFLQB16	Y87146	WO200004140-A1	Cancer
HAGFY16	Y87147	WO200004140-A1	Cancer
HMHBN40	Y87149	WO200004140-A1	Cancer
HDPBQ71	Y87150	WO200004140-A1	Cancer
HSKCT36	Y87151	WO200004140-A1	Cancer
HRACD80	Y87152	WO200004140-A1	Excretory, Reproductive
HSLCX03	Y87153	WO200004140-A1	Cancer
H2CBU83	Y87154	WO200004140-A1	Cancer
HFLQB16	Y87180	WO200004140-A1	Cancer
HAGFY16	Y87181	WO200004140-A1	Cancer
HFLQB16	Y87183	WO200004140-A1	Cancer
HAGFY16	Y87184	WO200004140-A1	Cancer
HMHBN40	Y87187	WO200004140-A1	Cancer
HDPBQ71	Y87188	WO200004140-A1	Cancer
HSKCT36	Y87192	WO200004140-A1	Cancer
HRACD80	Y87205	WO200004140-A1	Excretory, Reproductive
HSLCX03	Y87208	WO200004140-A1	Cancer
H2CBU83	Y87215	WO200004140-A1	Cancer
HISCH47	Y87784	US6054289-A	Cancer
HADCD24	Y87789	US6054289-A	Cancer
HDTEA84	Y90357	WO200052028-A1	Cancer
HKGCN17	Y91346	WO200011014-A1	Immune/Hematopoietic,

			Neural/Sensory, Reproductive
HETAD68	Y91347	WO200011014-A1	Cancer
HPIAT78	Y91348	WO200011014-A1	Cancer
HHFHG52	Y91349	WO200011014-A1	Cancer
HDTAB58	Y91350	WO200011014-A1	Cancer
HEOMQ62	Y91351	WO200011014-A1	Cancer
HWLJQ88	Y91352	WO200011014-A1	Digestive
HMICP03	Y91353	WO200011014-A1	Cancer
HAJAB01	Y91354	WO200011014-A1	Cancer
HE2AT09	Y91355	WO200011014-A1	Cancer
HSDJA15	Y91356	WO200011014-A1	Cancer
HAMGW29	Y91357	WO200011014-A1	Cancer
HAPSR85	Y91358	WO200011014-A1	Digestive, Endocrine
HTOHD42	Y91359	WO200011014-A1	Immune/Hematopoietic
HWLIH65	Y91360	WO200011014-A1	Cancer
HTOJA73	Y91361	WO200011014-A1	Immune/Hematopoietic
HPMGJ45	Y91362	WO200011014-A1	Cancer
HFVIC62	Y91363	WO200011014-A1	Digestive, Immune/Hematopoietic, Reproductive
HHENW77	Y91364	WO200011014-A1	Cancer
HMSIV91	Y91365	WO200011014-A1	Cancer
HMSKC04	Y91366	WO200011014-A1	Immune/Hematopoietic
HSAGZ33	Y91367	WO200011014-A1	Immune/Hematopoietic
HTEBC92	Y91368	WO200011014-A1	Cancer
HTXEL29	Y91369	WO200011014-A1	Immune/Hematopoietic
HDPAW44	Y91370	WO200011014-A1	Cancer
HMACS20	Y91371	WO200011014-A1	Cancer
HAJAY88	Y91372	WO200011014-A1	Immune/Hematopoietic
HBOEG69	Y91373	WO200011014-A1	Cancer
HWLEQ37	Y91374	WO200011014-A1	Cancer
HE9CS37	Y91375	WO200011014-A1	Cancer
HNGEI34	Y91376	WO200011014-A1	Immune/Hematopoietic
HTOAT76	Y91377	WO200011014-A1	Excretory, Immune/Hematopoietic
HDPVH60	Y91378	WO200011014-A1	Cancer
HLYCR65	Y91379	WO200011014-A1	Cancer
HARAY91	Y91380	WO200011014-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HCHNT03	Y91381	WO200011014-A1	Digestive, Reproductive
HCUBW95	Y91382	WO200011014-A1	Immune/Hematopoietic, Neural/Sensory
HDPLV95	Y91383	WO200011014-A1	Immune/Hematopoietic, Reproductive
HEMGB12	Y91384	WO200011014-A1	Cancer
HHENP27	Y91385	WO200011014-A1	Cancer
HSPBF70	Y91386	WO200011014-A1	Cancer
HTXKB57	Y91387	WO200011014-A1	Cancer
HUKAA55	Y91388	WO200011014-A1	Digestive, Immune/Hematopoietic, Reproductive
HFXGT58	Y91389	WO200011014-A1	Neural/Sensory

HUSFF19	Y91391	WO200011014-A1	Cancer
HUVDJ43	Y91393	WO200011014-A1	Cardiovascular, Reproductive
HTLCU49	Y91394	WO200011014-A1	Cancer
HDTAB58	Y91395	WO200011014-A1	Cancer
HWLJQ88	Y91396	WO200011014-A1	Digestive
HUVDJ43	Y91399	WO200011014-A1	Cardiovascular, Reproductive
HUVDJ43	Y91400	WO200011014-A1	Cardiovascular, Reproductive
HTLCU49	Y91401	WO200011014-A1	Cancer
HUVDJ43	Y91446	WO200011014-A1	Cardiovascular, Reproductive
HUVDJ43	Y91448	WO200011014-A1	Cardiovascular, Reproductive
HFKIB49	Y91449	WO200011014-A1	Cancer
HDPTK41	Y91451	WO200006698-A1	Cancer
HFXGT26	Y91452	WO200006698-A1	Cancer
HLTGX30	Y91453	WO200006698-A1	Immune/Hematopoietic
HLTHG37	Y91454	WO200006698-A1	Cancer
HNTMZ90	Y91455	WO200006698-A1	Digestive, Reproductive
HPIBX03	Y91456	WO200006698-A1	Cancer
H6EDY30	Y91457	WO200006698-A1	Cancer
HAMGR28	Y91458	WO200006698-A1	Cancer
HAPNZ94	Y91459	WO200006698-A1	Cancer
HATCP77	Y91460	WO200006698-A1	Cancer
HDABR72	Y91461	WO200006698-A1	Cancer
HDPKB18	Y91462	WO200006698-A1	Immune/Hematopoietic
HEQCC55	Y91463	WO200006698-A1	Cancer
HETDE26	Y91464	WO200006698-A1	Cancer
HOEDH84	Y91465	WO200006698-A1	Cancer
HPIBT55	Y91466	WO200006698-A1	Cancer
HSLCS05	Y91467	WO200006698-A1	Cancer
HDPDD03	Y91468	WO200006698-A1	Cancer
HDTDQ23	Y91470	WO200006698-A1	Cancer
HE2PY40	Y91471	WO200006698-A1	Mixed Fetal
HEONM66	Y91472	WO200006698-A1	Immune/Hematopoietic
HKAEG43	Y91473	WO200006698-A1	Cancer
HLHDP65	Y91474	WO200006698-A1	Cancer
HLMDO03	Y91475	WO200006698-A1	Cancer
HMAGK93	Y91476	WO200006698-A1	Cancer
HMEAL02	Y91477	WO200006698-A1	Cardiovascular
HMKCH52	Y91478	WO200006698-A1	Neural/Sensory
HCEFB69	Y91479	WO200006698-A1	Cancer
HKADM92	Y91480	WO200006698-A1	Cancer
HSPMG77	Y91481	WO200006698-A1	Digestive
HSQAC69	Y91482	WO200006698-A1	Cancer
HSTBJ86	Y91483	WO200006698-A1	Connective/Epithelial
HUVDJ43	Y91485	WO200006698-A1	Cardiovascular, Reproductive
HADCP14	Y91486	WO200006698-A1	Connective/Epithelial
HBXCF95	Y91487	WO200006698-A1	Cancer
HEQBU15	Y91488	WO200006698-A1	Cancer
HL1BD22	Y91489	WO200006698-A1	Cancer
HOEEU24	Y91490	WO200006698-A1	Cancer

HTTBR96	Y91491	WO200006698-A1	Reproductive
HWHQS55	Y91492	WO200006698-A1	Cancer
HCEEK50	Y91493	WO200006698-A1	Cancer
HCWBU94	Y91494	WO200006698-A1	Immune/Hematopoietic
HE2NR62	Y91495	WO200006698-A1	Cancer
HHSGL19	Y91496	WO200006698-A1	Neural/Sensory
HDPGT01	Y91497	WO200006698-A1	Cancer
HOHCA35	Y91499	WO200006698-A1	Cancer
HPMGP24	Y91500	WO200006698-A1	Mixed Fetal, Reproductive
HSDIE16	Y91501	WO200006698-A1	Neural/Sensory
HSOBK48	Y91502	WO200006698-A1	Digestive
HTADH39	Y91503	WO200006698-A1	Cancer
HUSGT36	Y91504	WO200006698-A1	Cardiovascular
HVAAE95	Y91505	WO200006698-A1	Digestive
HHEAH25	Y91506	WO200006698-A1	Cancer
HBJIY92	Y91507	WO200006698-A1	Cancer
HCLCW50	Y91508	WO200006698-A1	Respiratory
HDRMF68	Y91509	WO200006698-A1	Digestive, Respiratory
HOUGG12	Y91510	WO200006698-A1	Cancer
HEEAQ11	Y91511	WO200006698-A1	Reproductive
HEEAZ65	Y91512	WO200006698-A1	Musculoskeletal, Reproductive
HEGAN94	Y91513	WO200006698-A1	Reproductive
HFXBL33	Y91514	WO200006698-A1	Cancer
HLIBD68	Y91515	WO200006698-A1	Cancer
HLTCO33	Y91516	WO200006698-A1	Immune/Hematopoietic, Neural/Sensory, Reproductive
HLYAC95	Y91517	WO200006698-A1	Immune/Hematopoietic
HNHKS18	Y91519	WO200006698-A1	Immune/Hematopoietic
HSLJW78	Y91520	WO200006698-A1	Musculoskeletal
HHFHD01	Y91521	WO200006698-A1	Cardiovascular, Musculoskeletal, Neural/Sensory
HLWAE11	Y91522	WO200006698-A1	Cancer
HCYBN55	Y91523	WO200006698-A1	Cancer
HEONX38	Y91524	WO200006698-A1	Cancer
HLDQU79	Y91525	WO200006698-A1	Cancer
HSYBK21	Y91526	WO200006698-A1	Cancer
HTHDS25	Y91528	WO200006698-A1	Endocrine, Immune/Hematopoietic
HFIHO70	Y91529	WO200006698-A1	Cancer
HPMEI86	Y91530	WO200006698-A1	Cancer
HSOBV29	Y91531	WO200006698-A1	Cancer
HWABY10	Y91532	WO200006698-A1	Cancer
HACCI17	Y91533	WO200006698-A1	Cancer
HAPQT22	Y91534	WO200006698-A1	Immune/Hematopoietic
HDPBO81	Y91535	WO200006698-A1	Digestive, Immune/Hematopoietic, Reproductive
HDPGI49	Y91536	WO200006698-A1	Cancer
HDTBV77	Y91537	WO200006698-A1	Cancer
HFIUE82	Y91538	WO200006698-A1	Cancer
HHEND31	Y91539	WO200006698-A1	Cancer

HKMND01	Y91540	WO200006698-A1	Excretory
HLDBI84	Y91541	WO200006698-A1	Cancer
HLTEK17	Y91542	WO200006698-A1	Cancer
HEBEJ18	Y91543	WO200006698-A1	Cancer
HMEAI48	Y91544	WO200006698-A1	Cardiovascular
HNHGN91	Y91545	WO200006698-A1	Digestive, Endocrine, Immune/Hematopoietic
HODAE92	Y91546	WO200006698-A1	Cancer
HODDF13	Y91547	WO200006698-A1	Reproductive
HATEF60	Y91548	WO200006698-A1	Cancer
HLTHG37	Y91549	WO200006698-A1	Cancer
HAMGR28	Y91550	WO200006698-A1	Cancer
HDPKB18	Y91551	WO200006698-A1	Immune/Hematopoietic
HEQCC55	Y91552	WO200006698-A1	Cancer
HEONM66	Y91554	WO200006698-A1	Immune/Hematopoietic
HKAEG43	Y91555	WO200006698-A1	Cancer
HLHDP65	Y91556	WO200006698-A1	Cancer
HOEEU24	Y91557	WO200006698-A1	Cancer
HHEAH25	Y91558	WO200006698-A1	Cancer
HCYBN55	Y91559	WO200006698-A1	Cancer
HEONX38	Y91560	WO200006698-A1	Cancer
HFIHO70	Y91561	WO200006698-A1	Cancer
HACCI17	Y91562	WO200006698-A1	Cancer
HDPBO81	Y91563	WO200006698-A1	Digestive, Immune/Hematopoietic, Reproductive
HAMGR28	Y91599	WO200006698-A1	Cancer
HDPKB18	Y91603	WO200006698-A1	Immune/Hematopoietic
HEQCC55	Y91604	WO200006698-A1	Cancer
HLHDP65	Y91631	WO200006698-A1	Cancer
HOEEU24	Y91643	WO200006698-A1	Cancer
HHEAH25	Y91647	WO200006698-A1	Cancer
HHEAH25	Y91648	WO200006698-A1	Cancer
HLIBD68	Y91656	WO200006698-A1	Cancer
HCYBN55	Y91670	WO200006698-A1	Cancer
HEONX38	Y91672	WO200006698-A1	Cancer
HFIHO70	Y91679	WO200006698-A1	Cancer
HMKBA64	Y91681	WO200006698-A1	Cancer
HACCI17	Y91683	WO200006698-A1	Cancer
HMKEA94	Y93650	WO200036105-A1	Cancer
HE9SF68	Y93973	WO200042189-A1	Cancer
HTSGS30	Y93974	WO200042189-A1	Digestive, Immune/Hematopoietic, Mixed Fetal
HDQAC88	Y95534	WO200040726-A1	Cancer
HDPMM34	Y96280	WO200028035-A1	Cancer
HKABZ65	Y96962	WO200039327-A1	Connective/Epithelial
HWHGB15	Y96963	WO200039327-A1	Connective/Epithelial
HCDDP40	Y96964	WO200039327-A1	Immune/Hematopoietic, Musculoskeletal
HETBE01	B03767	US6066724-A	Cancer
HETGI70	B03768	US6066724-A	Cancer
HETDK42	B03769	US6066724-A	Cancer
HTEMZ33	B07705	WO200043493-A2	Cancer
HE8AW20	B07941	US6103871-A	Cancer



HNEDU15	B08659	WO200050597-A2	Cancer
HLTBT71	B08661	WO200050597-A2	Cancer
HBICD95	B08785	WO200050620-A2	Cancer
HE9CC44	B08786	US6110893-A	Cancer
HPRCC57	B10293	US6077692-A	Cancer
HPRCC57	B10304	US6077692-A	Cancer
HPRCC57	B10310	US6077692-A	Cancer
HPRCC57	B10311	US6077692-A	Cancer
HPRCC57	B10312	US6077692-A	Cancer
HPRCC57	B10313	US6077692-A	Cancer
HPRCC57	B10316	US6077692-A	Cancer
HPRCC57	B10320	US6077692-A	Cancer
HILBX90	B11125	US6133422-A	Cancer
HCQAS17	B12900	US6080722-A	Digestive, Mixed Fetal, Reproductive
HBMSE33	B15366	WO200042165-A2	Cancer
HE2BG16	B15413	US6090575-A	Cancer
HT4CC72	B18618	WO200053223-A1	Immune/Hematopoietic
HAPOR40	B18750	WO200055204-A1	Cancer
HTSGS30	B18755	WO200055204-A1	Digestive, Immune/Hematopoietic, Mixed Fetal
HFGAM58	B18803	WO200053210-A1	Cancer
HSDFB55	B19550	WO200053793-A1	Cancer
HUVEO91	B19863	WO200066608-A1	Cancer
HCDDP40	B25583	WO200029435-A1	Immune/Hematopoietic, Musculoskeletal
HMELK96	B26981	WO200056862-A1	Cancer
HMELK96	B26987	WO200056862-A1	Cancer
HTTBN61	B26990	WO200056862-A1	Cancer
HCUDS60	B26991	WO200056862-A1	Cancer
HLYBX88	B26992	WO200056862-A1	Cancer
HILBI36	B28524	US6130051-A	Cancer
HLYBX88	B29790	WO200066156-A1	Cancer
HCEMP60	B29923	US6130061-A	Cancer
HE8AE45	B33821	WO200056753-A1	Cancer
HE2OA95	B33822	WO200056753-A1	Cancer
HJACE54	B35705	WO200063221-A2	Cancer
HTTBN61	B36265	WO200064465-A1	Cancer
HPRCB54	B36696	WO200071150-A1	Cancer
HSDME38	B39392	WO200057903-A2	Cancer
HSDME38	B39393	WO200057903-A2	Cancer
HPDDY64	B43604	WO200055350-A1	Cancer
HPABA51	B44685	WO200058339-A2	Cancer
HPMSM24	B45376	WO200061628-A1	Cancer
HOUCQ17	B50002	WO200071577-A1	Cancer
HODAH63	B50272	WO200071567-A2	Neural/Sensory, Reproductive
HODAH63	B50282	WO200071567-A2	Neural/Sensory, Reproductive
HODAH63	B50283	WO200071567-A2	Neural/Sensory, Reproductive
HCEGY95	B50289	WO200071582-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory

HE9CC44	B50293	WO200071715-A1	Cancer
HAGAT55	B50294	WO200071715-A1	Cancer
HAGAT55	B50704	WO200071152-A1	Cancer
HKABO35	B50892	WO200073321-A1	Cancer
HETJY78	B51152	US6153739-A	Cancer
HPRCC57	B58248	WO200055180-A2	Cancer
HHFCU19	B58276	WO200055180-A2	Cancer
HNFA09	B58319	WO200055180-A2	Cancer
HBZSD43	B58925	WO200055173-A1	Cancer
HHFHJ57	B58970	WO200055173-A1	Cancer
HPRCC57	B60201	WO200072872-A1	Cancer
HPRCC57	B60204	WO200072872-A1	Cancer
HPRCC57	B60206	WO200072872-A1	Cancer
HPRCC57	B60207	WO200072872-A1	Cancer
HPRCC57	B60208	WO200072872-A1	Cancer
HPRCC57	B60209	WO200072872-A1	Cancer
HPRCC57	B60210	WO200072872-A1	Cancer
HPRCC57	B60212	WO200072872-A1	Cancer
HPRCC57	B60214	WO200072872-A1	Cancer
HRGBQ38	B64643	WO200077197-A1	Cancer
HRGBQ38	B64644	WO200077197-A1	Cancer
HRGBQ38	B64645	WO200077197-A1	Cancer
HRGBQ38	B64646	WO200077197-A1	Cancer
HLTBT71	B64873	WO200077256-A1	Cancer
HTEIX55	B64953	WO200076530-A1	Cancer
HPABA51	R75085	ZA9403789-A	Cancer
HAPAT57	R76127	WO9517092-A	Cancer
HWFBD68	R76128	WO9517092-A	Cancer
HMP5A79	R77649	WO9532282-A1	Cancer
HGBAB73	R79008	WO9520678-A1	Cancer
HLTAW73	R79009	WO9520678-A1	Cancer
HFCAW19	R80095	WO9527781-A1	Cancer
HHFBT80	R80575	WO9524474-A1	Cancer
HFKCU96	R81309	WO9519985-A1	Cancer
HSRAW34	R81461	WO9605226-A1	Cancer
HOSBD47	R82686	WO9524473-A1	Cancer
HOSBH74	R82720	WO9524182-A1	Cancer
HE8AE45	R82987	WO9524466-A1	Cancer
HLFBE10	R84522	WO9524411-A1	Cancer
HAGAT55	R85650	WO9524414-A1	Cancer
HIBEC52	R87954	WO9530428-A1	Cancer
HTEAH87	R88390	WO9531539-A1	Cancer
HFBEH64	R88405	WO9531538-A1	Cancer
HAFAK86	R88419	WO9535372-A1	Cancer
HASSB35	R88452	WO9600242-A1	Cancer
HPAAA47	R88481	WO9601270-A1	Cancer
HJPAH22	R90703	WO9600297-A1	Cancer
HIBCL76	R90764	WO9603415-A1	Cancer
HIBEJ89	R90765	WO9603415-A1	Neural/Sensory
HLFBE49	R90919	WO9601896-A	Cancer
HIBCL22	R90989	WO9605225-A1	Cancer
HSSAW84	R91929	WO9612791-A1	Cancer
HSNME29	R92220	WO9604928-A1	Cancer
HSNME29	R92753	WO9605221-A	Cancer
HGBAN46	R93086	WO9605856-A1	Cancer

HE9DR66	R93087	WO9605856-A1	Cancer
HTPAN40	R93118	WO9606862-A	Cancer
HILBI36	R93156	WO9608557-A1	Cancer
HJBAQ29	R94350	WO9609311-A1	Cancer
HASAC73	R94601	WO9611259-A1	Cancer
HPLAP22	R94602	WO9611259-A1	Cancer
HT2SA16	R95634	WO9614394-A1	Cancer
HLHAC42	R95692	WO9615806-A1	Cancer
HE2CA82	R95830	WO9613603-A1	Cancer
HTOBA30	R95831	WO9613603-A1	Cancer
HFSBE16	R97222	WO9616087-A1	Cancer
HHFCU19	R97565	WO9621736-A1	Cancer
HE8AW20	R97739	WO9615222-A1	Cancer
HSBBC75	R97978	WO9615147-A1	Cancer
HLFBG09	R98224	WO9612501-A1	Cancer
HHPEC49	R98261	WO9611946-A1	Cancer
HFGAM58	R98265	WO9618725-A1	Cancer
HUVCT01	R98994	WO9617931-A1	Cancer
HFSAG79	R99329	WO9624668-A1	Cancer
HATBG78	R99353	WO9627009-A1	Endocrine
HUVEO91	R99453	WO9614328-A1	Cancer
HPRCC57	W00176	WO9625422-A1	Cancer
HCAAA02	W00482	WO9621724-A1	Cancer
HETAN67	W01097	WO9629401-A1	Cancer
HSSNB01	W01098	WO9629401-A1	Cancer
HETJY78	W01619	WO9635778-A1	Cancer
HPRAJ70	W01730	WO9639435-A1	Cancer
HNFAQ09	W02151	WO9625432-A1	Cancer
HFSBC65	W02613	WO9618730-A1	Cancer
HE2BG16	W04247	WO9630406-A1	Cancer
HTECE68	W05295	WO9630524-A1	Cancer
HRGBQ38	W05313	WO9623410-A1	Cancer
HFCCE09	W05314	WO9623410-A1	Cancer
HGOCA18	W05315	WO9623410-A1	Cancer
HT1SB52	W05809	WO9634095-A1	Cancer
HFGAN72	W06124	WO9634877-A1	Cancer
HHFBT80	W06539	WO9639431-A1	Cancer
HCNAY46	W06545	WO9639419-A1	Cancer
HCQDM23	W06546	WO9639419-A1	Digestive, Reproductive
HCNUB65	W06548	WO9639419-A1	Cancer
HCNSE58	W06550	WO9639419-A1	Cancer
HCNBB33	W06551	WO9639419-A1	Cancer
HKLSA58	W06552	WO9639419-A1	Cancer
HCNSD13	W06553	WO9639419-A1	Cancer
HLQBI14	W06575	WO9639520-A1	Cancer
HAECD08	W07202	WO9634891-A1	Cancer
HWFBD68	W07203	WO9634891-A1	Cancer
HAPAT57	W07204	WO9634891-A1	Cancer
HDGNR10	W07602	WO9639437-A1	Digestive, Immune/Hematopoietic, Reproductive
HMSDB49	W07604	WO9639521-A1	Immune/Hematopoietic, Reproductive
HFSAG79	W07605	WO9639522-A1	Cancer

HTOEX74	W07606	WO9639522-A1	Cancer
HMWCF06	W07611	WO9639421-A1	Cancer
HIBEB69	W07617	WO9639438-A1	Cancer
HGBER32	W07618	WO9639434-A1	Digestive
HETGQ23	W07619	WO9639436-A1	Cancer
HE2OA95	W07663	WO9636709-A1	Cancer
HCEGY95	W08079	WO9639506-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HIBEF51	W08101	WO9639441-A1	Neural/Sensory
HTNAD29	W08141	WO9639442-A1	Cancer
HE9CC44	W08142	WO9639507-A1	Cancer
HDGRC02	W09110	WO9639440-A1	Cancer
HPTTT24	W09111	WO9639420-A1	Digestive, Endocrine
HUVDR03	W09404	WO9639485-A1	Cancer
HPBCB95	W09405	WO9639158-A1	Cancer
HE9NG77	W09408	WO9639486-A1	Cancer
HATCK89	W09432	WO9639509-A1	Cancer
HTOEX74	W10574	WO9624668-A1	Cancer
HOSBD47	W11478	WO9639515-A1	Cancer
HCQAS17	W12691	WO9639541-A1	Digestive, Mixed Fetal, Reproductive
HCACU62	W12692	WO9639424-A1	Cancer
HAQBM60	W12693	WO9639418-A1	Connective/Epithelial, Immune/Hematopoietic, Reproductive
HLTDG74	W12695	WO9639433-A1	Immune/Hematopoietic
HODAH63	W12696	WO9639508-A1	Neural/Sensory, Reproductive
HSSAW84	W17043	US5618717-A	Cancer
HHSAN40	W17838	WO9717358-A1	Cancer
HTPBS22	W19632	WO9722623-A1	Cancer
HSATU68	W19780	WO9725340-A1	Cancer
HFCBS02	W22408	WO9711970-A1	Cancer
HGBAN46	W22669	WO9731098-A1	Cancer
HE9DR66	W22670	WO9731098-A1	Cancer
HE9DR66	W22671	WO9731098-A1	Cancer
HE9DR66	W22672	WO9731098-A1	Cancer
HE9DR66	W22673	WO9731098-A1	Cancer
HE9DR66	W22674	WO9731098-A1	Cancer
HE9DR66	W22675	WO9731098-A1	Cancer
HPMSM24	W22732	WO9724929-A1	Cancer
HPABA51	W22882	US5635616-A	Cancer
HESAJ20	W23663	WO9729189-A1	Cancer
HALTA54	W24137	WO9723640-A1	Cancer
HCEMP60	W24847	WO9718224-A1	Cancer
HFCCE09	W25112	US5650313-A	Cancer
HGOCA18	W25113	US5650313-A	Cancer
HRGBQ38	W25114	US5650313-A	Cancer
HTPAN40	W26464	US5654172-A	Cancer
HTECD31	W27087	WO9725349-A1	Cancer
HLHDC84	W27118	WO9725338-A1	Cancer
HTPAN08	W27134	WO9733899-A1	Cancer
HFKET35	W27152	WO9734013-A1	Cancer

HTTER36	W27224	WO9735870-A1	Cardiovascular, Connective/Epithelial, Reproductive
HTOBH93	W27561	WO9727747-A1	Cancer
HE6BK61	W29291	WO9735010-A1	Cancer
HE6BK61	W29292	WO9735010-A1	Cancer
HTOJK64	W30193	WO9735976-A2	Cancer
HLMBA70	W30891	WO9735028-A1	Immune/Hematopoietic, Mixed Fetal, Reproductive
HMEIP65	W31512	WO9732993-A1	Cancer
HTTBN61	W31517	WO9733904-A1	Cancer
HFLQA68	W31527	WO9737022-A1	Cancer
HE8AW20	W31692	US5695980-A	Cancer
HTXEI33	W31759	WO9733898-A1	Cancer
HCUDE60	W31902	WO9737021-A1	Cancer
HCABA58	W32110	WO9738012-A1	Cancer
HMEAN51	W32112	WO9734998-A1	Cancer
HT4CC72	W32255	WO9734911-A1	Immune/Hematopoietic
HCUDE60	W32323	WO9736915-A1	Cancer
HSAAU35	W33603	WO9747742-A1	Connective/Epithelial, Musculoskeletal, Reproductive
HETBE01	W35802	WO9734997-A1	Cancer
HETGI70	W35803	WO9734997-A1	Cancer
HETDK42	W35804	WO9734997-A1	Cancer
HBJEL88	W35904	WO9738003-A1	Cancer
HSCL68	W36449	WO9735027-A1	Cancer
HLTBT71	W37002	WO9733902-A1	Cancer
HPDDO12	W37003	WO9733902-A1	Cancer
HHPEC49	W37799	US5750370-A	Cancer
HCQAJ72	W37844	WO9807749-A1	Cancer
HMECG71	W37845	WO9807749-A1	Cancer
HSIEH63	W37846	WO9807749-A1	Digestive
HBICD95	W37847	WO9807880-A1	Cancer
HMQBM23	W37935	WO9808870-A1	Cancer
HOEBG39	W37946	WO9821236-A1	Cancer
HOSBH74	W39216	EP812916-A2	Cancer
HOSBH74	W39264	EP812916-A2	Cancer
HOSBH74	W39265	EP812916-A2	Cancer
HOSBH74	W39266	EP812916-A2	Cancer
HOSBH74	W39267	EP812916-A2	Cancer
HOSBH74	W39268	EP812916-A2	Cancer
HODAH63	W40077	US5728546-A	Neural/Sensory, Reproductive
HFBEH64	W41362	US5723311-A	Cancer
HSAAU35	W41502	EP812913-A2	Connective/Epithelial, Musculoskeletal, Reproductive
HSAAU35	W41520	WO9747741-A1	Connective/Epithelial, Musculoskeletal, Reproductive
HOSBH74	W41645	WO9747642-A1	Cancer
HTSEX82	W41938	WO9748807-A1	Digestive, Immune/Hematopoietic
HIBCL76	W42995	US5710019-A	Cancer

HIBEJ89	W42996	US5710019-A	Neural/Sensory
HILBI36	W46518	US5716806-A	Cancer
HCNAY46	W46876	US5733748-A	Cancer
HCQDM23	W46877	US5733748-A	Digestive, Reproductive
HCNUB65	W46879	US5733748-A	Cancer
HCNSE58	W46882	US5733748-A	Cancer
HCNBB33	W46883	US5733748-A	Cancer
HKLSA58	W46884	US5733748-A	Cancer
HCNSD13	W46885	US5733748-A	Cancer
HEMEM90	W48334	WO9807881-A1	Cancer
HE9BK24	W48335	WO9807754-A1	Cancer
HPASD50	W48391	WO9807735-A1	Cancer
HETAN67	W48762	WO9812204-A1	Cancer
HHFHJ57	W49032	WO9825957-A2	Cancer
HGBER32	W49807	US5776729-A	Digestive
HATCK89	W49826	US5773252-A	Cancer
HCEPR64	W51244	WO9821242-A1	Cancer
HPRCC57	W52581	WO9806844-A1	Cancer
HPRCC57	W52582	WO9806844-A1	Cancer
HPRCC57	W52583	WO9806844-A1	Cancer
HPRCC57	W52584	WO9806844-A1	Cancer
HPRCC57	W52585	WO9806844-A1	Cancer
HPRCC57	W52586	WO9806844-A1	Cancer
HPRCC57	W52587	WO9806844-A1	Cancer
HPRCC57	W52588	WO9806844-A1	Cancer
HPRCC57	W52590	WO9806844-A1	Cancer
HPRCC57	W52591	WO9806844-A1	Cancer
HPRCC57	W52592	WO9806844-A1	Cancer
HPRCC57	W52593	WO9806844-A1	Cancer
HPRCC57	W52594	WO9806844-A1	Cancer
HPRCC57	W52595	WO9806844-A1	Cancer
HPRCC57	W52596	WO9806844-A1	Cancer
HPRCC57	W52597	WO9806844-A1	Cancer
HPRCC57	W52598	WO9806844-A1	Cancer
HPRCC57	W52599	WO9806844-A1	Cancer
HDQMB53	W52842	WO9807862-A2	Cancer
HWFBF68	W52843	WO9807862-A2	Cancer
HPMFW51	W53121	WO9806859-A1	Cancer
HPMFW51	W53122	WO9806859-A1	Cancer
HPRCC57	W53787	WO9806844-A1	Cancer
HPRCC57	W53792	WO9806844-A1	Cancer
HPRCC57	W53793	WO9806844-A1	Cancer
HMEEJ22	W53897	WO9808969-A1	Cancer
HE9CC44	W54036	US5763214-A	Cancer
HMQCD14	W55884	WO9806733-A1	Cancer
HUVDE75	W56249	WO9806839-A1	Cancer
HCNBB33	W56503	WO9815624-A1	Cancer
HTPBR22	W56504	WO9815624-A1	Cancer
HETAS87	W56505	WO9815624-A1	Cancer
HETAS87	W56506	WO9815624-A1	Cancer
HPRAJ70	W56641	US5756309-A	Cancer
HAICL46	W57044	WO9811138-A1	Cancer
HAECD08	W57688	WO9814582-A1	Cancer
HAECD08	W57691	WO9814582-A1	Cancer

HAECD08	W57692	WO9814582-A1	Cancer
HAECD08	W57693	WO9814582-A1	Cancer
HAECD08	W57694	WO9814582-A1	Cancer
HAECD08	W57695	WO9814582-A1	Cancer
HWFBD68	W57697	WO9814582-A1	Cancer
HAPAT57	W57698	WO9814582-A1	Cancer
HAECD08	W57699	WO9814582-A1	Cancer
HAECD08	W57701	WO9814582-A1	Cancer
HMSDB49	W57881	WO9824908-A1	Immune/Hematopoietic, Reproductive
HNEDU15	W58391	WO9818921-A1	Cancer
HE9NG77	W58704	US5780263-A	Cancer
HFSAG79	W58900	WO9814477-A1	Cancer
HTOEX74	W58901	WO9814477-A1	Cancer
HTOEX74	W58902	WO9814477-A1	Cancer
HTOEX74	W58903	WO9814477-A1	Cancer
HTOEX74	W58904	WO9814477-A1	Cancer
HTOEX74	W58905	WO9814477-A1	Cancer
HTOEX74	W58906	WO9814477-A1	Cancer
HTOEX74	W58907	WO9814477-A1	Cancer
HTOEX74	W58908	WO9814477-A1	Cancer
HTOEX74	W58909	WO9814477-A1	Cancer
HTOEX74	W58910	WO9814477-A1	Cancer
HTOEX74	W58911	WO9814477-A1	Cancer
HTOEX74	W58912	WO9814477-A1	Cancer
HTOEX74	W58913	WO9814477-A1	Cancer
HTOEX74	W58914	WO9814477-A1	Cancer
HTOEX74	W58915	WO9814477-A1	Cancer
HTOEX74	W58916	WO9814477-A1	Cancer
HTOEX74	W58917	WO9814477-A1	Cancer
HTOEX74	W58918	WO9814477-A1	Cancer
HTOEX74	W58919	WO9814477-A1	Cancer
HTOEX74	W58920	WO9814477-A1	Cancer
HTOEX74	W58921	WO9814477-A1	Cancer
HTOEX74	W58922	WO9814477-A1	Cancer
HTOEX74	W58923	WO9814477-A1	Cancer
HTOEX74	W58924	WO9814477-A1	Cancer
HTOEX74	W58925	WO9814477-A1	Cancer
HTOEX74	W58926	WO9814477-A1	Cancer
HTOEX74	W58927	WO9814477-A1	Cancer
HTOEX74	W58928	WO9814477-A1	Cancer
HTOEX74	W58929	WO9814477-A1	Cancer
HTOEX74	W58930	WO9814477-A1	Cancer
HTOEX74	W58931	WO9814477-A1	Cancer
HTOEX74	W58932	WO9814477-A1	Cancer
HTOEX74	W58933	WO9814477-A1	Cancer
HTOEX74	W58934	WO9814477-A1	Cancer
HTOEX74	W58935	WO9814477-A1	Cancer
HTOEX74	W58936	WO9814477-A1	Cancer
HTOEX74	W58937	WO9814477-A1	Cancer
HTOEX74	W58938	WO9814477-A1	Cancer
HTOEX74	W58939	WO9814477-A1	Cancer
HTOEX74	W58940	WO9814477-A1	Cancer
HTOEX74	W58941	WO9814477-A1	Cancer
HFSAG79	W58942	WO9814477-A1	Cancer

HFSAG79	W58943	WO9814477-A1	Cancer
HFSAG79	W58944	WO9814477-A1	Cancer
HFSAG79	W58945	WO9814477-A1	Cancer
HFSAG79	W58946	WO9814477-A1	Cancer
HFSAG79	W58947	WO9814477-A1	Cancer
HFSAG79	W58948	WO9814477-A1	Cancer
HFSAG79	W58949	WO9814477-A1	Cancer
HFSAG79	W58950	WO9814477-A1	Cancer
HFSAG79	W58951	WO9814477-A1	Cancer
HFSAG79	W58952	WO9814477-A1	Cancer
HFSAG79	W58953	WO9814477-A1	Cancer
HFSAG79	W58954	WO9814477-A1	Cancer
HFSAG79	W58955	WO9814477-A1	Cancer
HFSAG79	W58956	WO9814477-A1	Cancer
HFSAG79	W58957	WO9814477-A1	Cancer
HFSAG79	W58958	WO9814477-A1	Cancer
HFSAG79	W58959	WO9814477-A1	Cancer
HFSAG79	W58960	WO9814477-A1	Cancer
HFSAG79	W58961	WO9814477-A1	Cancer
HFSAG79	W58962	WO9814477-A1	Cancer
HFSAG79	W58963	WO9814477-A1	Cancer
HFSAG79	W58964	WO9814477-A1	Cancer
HFSAG79	W58965	WO9814477-A1	Cancer
HFSAG79	W58966	WO9814477-A1	Cancer
HFSAG79	W58967	WO9814477-A1	Cancer
HFSAG79	W58968	WO9814477-A1	Cancer
HFSAG79	W58969	WO9814477-A1	Cancer
HFSAG79	W58970	WO9814477-A1	Cancer
HFSAG79	W58971	WO9814477-A1	Cancer
HFSAG79	W58972	WO9814477-A1	Cancer
HFSAG79	W58973	WO9814477-A1	Cancer
HFSAG79	W58974	WO9814477-A1	Cancer
HFSAG79	W58975	WO9814477-A1	Cancer
HCEGH45	W59666	WO9824900-A1	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HHFCU19	W59753	US5786193-A	Cancer
HLMBP36	W59872	WO9831792-A1	Cancer
HEMFI85	W59873	WO9831800-A2	Cancer
HTXET53	W59874	WO9831800-A2	Cancer
HBZAK03	W59876	WO9831800-A2	Cancer
HLFBD44	W59877	WO9831800-A2	Cancer
HEBGM49	W59878	WO9831800-A2	Cancer
HNGBH54	W59879	WO9831800-A2	Cancer
HSAAL25	W59880	WO9831800-A2	Cancer
HSXCK41	W59882	WO9831800-A2	Cancer
HFKFY79	W59883	WO9831800-A2	Cancer
HAICH28	W59884	WO9831800-A2	Cancer
HT1SB52	W60045	WO9818824-A1	Cancer
HSDFB55	W60054	WO9816643-A1	Cancer
HEBBC23	W60607	WO9820110-A1	Immune/Hematopoietic, Musculoskeletal, Neural/Sensory
HTPBS22	W61600	WO9831798-A1	Cancer
HMACR70	W61616	WO9831799-A2	Cancer



HTEDK48	W61617	WO9831799-A2	Cancer
HTPEF86	W61619	WO9831799-A2	Cancer
HSBBF02	W61620	WO9831799-A2	Cancer
HLTAH80	W61621	WO9831799-A2	Cancer
HTPBA27	W61622	WO9831799-A2	Cancer
HAIDQ59	W61623	WO9831799-A2	Cancer
HHFEK40	W61624	WO9831799-A2	Cancer
HGBGV89	W61625	WO9831799-A2	Digestive
HUVBB80	W61626	WO9831799-A2	Cancer
HJACE54	W61627	WO9831799-A2	Cancer
HROAD63	W61628	WO9831799-A2	Connective/Epithelial, Digestive
HMWGS46	W61629	WO9831799-A2	Cancer
HNFGW06	W61630	WO9831799-A2	Cancer
HFCAR05	W61912	WO9820042-A1	Cancer
HHFHG78	W62595	WO9827932-A2	Cancer
HBGBA67	W63123	WO9833915-A1	Cancer
HPHAE52	W63622	WO9830694-A2	Cancer
HTPCH84	W63623	WO9830694-A2	Cancer
HEBCI67	W64433	WO9829438-A2	Cancer
HCUDS60	W64483	WO9832856-A1	Cancer
HPRCB54	W64668	WO9830693-A2	Cancer
HTOCD71	W69220	WO9828421-A1	Cancer
HSGSA61	W69221	WO9828420-A1	Cancer
HSLAZ11	W69229	WO9831801-A1	Cancer
HCEBJ50	W69230	WO9831801-A1	Cancer
HMQDO20	W69231	WO9831806-A2	Cancer
HDPMK33	W69232	WO9831806-A2	Cancer
HMPAP73	W69233	WO9831806-A2	Immune/Hematopoietic
HMSHH46	W69234	WO9831806-A2	Cancer
HMAAB68	W69235	WO9831806-A2	Digestive, Immune/Hematopoietic
HSDME38	W69508	WO9828422-A1	Cancer
HOEBN05	W70286	WO9833920-A2	Cancer
HDPMJ44	W70287	WO9835039-A1	Cancer
HODAH63	W70330	WO9823749-A1	Neural/Sensory, Reproductive
HETDW91	W70458	WO9838311-A1	Cancer
HE8CV92	W70459	WO9838311-A1	Cancer
HIBCL22	W70501	US5817477-A	Cancer
HKFBA76	W70525	WO9844111-A1	Cancer
HKFBA76	W70526	WO9844111-A1	Cancer
HMSAF34	W70594	WO9844118-A1	Cancer
HMSAF34	W70596	WO9844118-A1	Cancer
HMSAF34	W70597	WO9844118-A1	Cancer
HRDCD54	W71592	WO9833912-A1	Cancer
HIBEC52	W73130	US5830744-A	Cancer
HSRAW34	W73635	US5861272-A	Cancer
HBWAL95	W76212	WO9837194-A1	Cancer
HTEJQ70	W76251	WO9831818-A2	Cancer
HETBW05	W76253	WO9831818-A2	Digestive, Reproductive
HATBG78	W77493	US5798223-A	Endocrine
HMWGS46	W78168	WO9856804-A1	Cancer
HOUCQ17	W78189	WO9856804-A1	Cancer

HMWGS46	W78295	WO9856804-A1	Cancer
HLYBX88	W79083	WO9841629-A2	Cancer
HTAAW41	W80212	WO9844112-A1	Cancer
HOUCQ17	W80285	EP874050-A2	Cancer
HMELK96	W81059	WO9856892-A1	Cancer
HLJBI75	W81071	WO9851794-A1	Cancer
HFCBS02	W81106	WO9844109-A1	Cancer
HHPGS02	W81576	WO9850549-A2	Cancer
HTOBH93	W83929	US5844081-A	Cancer
HSSAE30	W84184	WO9853069-A2	Cancer
HCQAS17	W84274	US5861494-A	Digestive, Mixed Fetal, Reproductive
HRGBQ38	W85561	US5849286-A	Cancer
HFCCE09	W85562	US5849286-A	Cancer
HGOCA18	W85563	US5849286-A	Cancer
HMSIB42	W87769	WO9854199-A1	Cancer
HTECE68	W89575	US5858705-A	Cancer
HESAJ20	W92460	US5871969-A	Cancer
HESAJ20	W92469	US5871969-A	Cancer
HTXEI33	W92523	US5874240-A	Cancer
HTXEI33	W92524	US5874240-A	Cancer
HKABO35	W92792	WO9854202-A1	Cancer
HCEGH45	W94074	US5869632-A	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HNFIR05	W94466	WO9900415-A1	Cancer
HTTBN61	W95538	JP11000170-A	Cancer
HPFCA19	W96192	WO9900498-A1	Cancer
HPFCA19	W96193	WO9900498-A1	Cancer
HTSGS30	W97350	WO9903982-A1	Digestive, Immune/Hematopoietic, Mixed Fetal
HDTAH85	Y01098	WO9910364-A1	Cancer
HFJAB36	Y02608	WO9923106-A1	Cancer
HDTBS70	Y03231	WO9909152-A1	Cancer
HNGEF08	Y03849	WO9909198-A1	Immune/Hematopoietic, Reproductive
HUKEJ46	Y03850	WO9909198-A1	Digestive, Reproductive
HPASD50	Y04120	WO9909161-A1	Cancer
HPASD50	Y04121	WO9909161-A1	Cancer
HAGFE38	Y05451	WO9857989-A1	Cancer
HFVIF40	Y06461	WO9931116-A1	Cancer
HFCCQ50	Y06462	WO9931116-A1	Cancer
HT4CC72	Y06473	WO9935262-A2	Immune/Hematopoietic
HDPIE88	Y06511	WO9936565-A1	Cancer
HWFBG79	Y10797	WO9907891-A1	Cancer
HDGRC02	Y13736	US5928890-A	Cancer
HFCET92	Y14078	WO9921575-A1	Cancer
HUVEO91	Y14132	WO9923105-A1	Cancer
HUVEO91	Y14133	WO9923105-A1	Cancer
HCABA58	Y16587	US5916769-A	Cancer
HOSBD47	Y22320	US5932540-A	Cancer
HOSBD47	Y22321	US5932540-A	Cancer
HPRCC57	Y23761	WO9932135-A1	Cancer

HMEAA94	Y23884	WO9935160-A1	Cancer
HLIAP03	Y23885	WO9935160-A1	Cancer
HSYBM46	Y23886	WO9935160-A1	Cancer
HFKBC47	Y23887	WO9935160-A1	Cancer
HSSAW84	Y24249	US5929225-A	Cancer
HCUDE60	Y25708	WO9938882-A1	Cancer
HHFCU19	Y27005	US5928924-A	Cancer
HMWJH67	Y28640	WO9940183-A1	Cancer
HKAFV61	Y28642	WO9940183-A1	Cancer
HETDK50	Y28643	WO9940183-A1	Cancer
HKAEF09	Y28644	WO9940183-A1	Cancer
HOSBD47	Y30518	WO9946364-A1	Cancer
HOSBD47	Y30519	WO9946364-A1	Cancer
HILBI36	Y31242	US5955339-A	Cancer
HTAEK53	Y31810	WO9947538-A1	Cancer
HT4CC72	Y31885	WO9942584-A1	Immune/Hematopoietic
HLMBA70	Y32504	US5945309-A	Immune/Hematopoietic, Mixed Fetal, Reproductive
HPRCC57	Y32888	WO9941282-A1	Cancer
HPRCC57	Y32895	WO9941282-A1	Cancer
HPRCC57	Y32896	WO9941282-A1	Cancer
HPRCC57	Y32897	WO9941282-A1	Cancer
HPRCC57	Y32898	WO9941282-A1	Cancer
HPRCC57	Y32901	WO9941282-A1	Cancer
HPRCC57	Y32905	WO9941282-A1	Cancer
HPRCC57	Y32916	WO9941282-A1	Cancer
HMEIP65	Y33847	US5952197-A	Cancer
HFCCQ50	Y36339	WO9931117-A1	Cancer
HFCCQ50	Y36342	WO9931117-A1	Cancer
HRDCD54	Y36648	WO9931117-A1	Cancer
HRDCD54	Y36650	WO9931117-A1	Cancer
HRDCD54	Y36673	WO9931117-A1	Cancer
HTSEX82	Y41161	US5981231-A	Digestive, Immune/Hematopoietic
HGBAN46	Y41163	US5981230-A	Cancer
HE9DR66	Y41164	US5981230-A	Cancer
HRGBQ38	Y42150	US5968797-A	Cancer
HFCCE09	Y42151	US5968797-A	Cancer
HGOCA18	Y42152	US5968797-A	Cancer
HNFEM05	Y42165	WO9927078-A1	Cancer
HJACE54	Y44510	WO200001728-A1	Cancer
HKAEF92	Y44664	WO9962934-A1	Cancer
HBZSD43	Y45003	WO200006589-A1	Cancer
HUVEO91	Y45032	WO200008139-A1	Cancer
HTOBH93	Y49535	US5977309-A	Cancer
HAPOR40	Y49946	WO9914240-A1	Cancer
HHEAC71	Y52158	WO9920758-A1	Connective/Epithelial, Immune/Hematopoietic
HCFAZ22	Y52159	WO9920758-A1	Cancer
HT5EA78	Y52160	WO9920758-A1	Connective/Epithelial, Immune/Hematopoietic
HDPJO39	Y52479	WO9940184-A1	Cancer
HBICD95	Y53061	US5998171-A	Cancer
HTGED19	Y53890	WO9961617-A1	Immune/Hematopoietic

HFPBX96	Y53891	WO9961617-A1	Cancer
HFKCU96	Y54900	US5986069-A	Cancer
HSBBC75	Y55748	US5994103-A	Cancer
HLFBE10	Y55750	US5994103-A	Cancer
HLFBE10	Y57166	US5994301-A	Cancer
HIBCL22	Y57167	US5994506-A	Cancer
HTTER36	Y58185	US6004780-A	Cardiovascular, Connective/Epithelial, Reproductive
HWHGU74	Y59247	WO9962927-A1	Cancer
HSDFB55	Y67239	US6008020-A	Cancer
HE2BG16	Y67356	US5998164-A	Cancer
HKAPI15	Y68800	WO200005371-A1	Connective/Epithelial
HTWAF38	Y69674	US6013483-A	Cancer
HATCK89	Y69675	US6013477-A	Cancer
HAPOR40	Y70591	WO200015759-A1	Cancer
HMUAN45	Y70785	WO200023572-A1	Cancer
HATCK89	Y71884	WO200067775-A1	Cancer
HKGDL36	Y71959	WO200066778-A1	Cancer
HCUDS60	Y72022	WO200067793-A1	Cancer
HCUDS60	Y72023	WO200067793-A1	Cancer
HETAN67	Y78790	US6013469-A	Cancer
HDGNR10	Y80128	US6025154-A	Digestive, Immune/Hematopoietic, Reproductive
HBGBA67	Y87779	US6054289-A	Cancer
HE2CB95	Y87780	US6054289-A	Immune/Hematopoietic, Mixed Fetal
HPTTK55	Y87782	US6054289-A	Cancer
HARAO63	Y87783	US6054289-A	Cancer
HLHAR55	Y87787	US6054289-A	Cancer
HSRDG78	Y87788	US6054289-A	Cancer
HCCAA03	Y87789	US6054289-A	Cancer
HWLLM34	Y90351	WO200052136-A2	Cancer
HA5AA37	Y90352	WO200052136-A2	Cancer
HDPK85	Y90353	WO200052136-A2	Cancer
HPHAE52	Y90357	WO200052028-A1	Cancer
HTPCH84	Y90358	WO200052028-A1	Cancer
HMKEA94	Y93650	WO200036105-A1	Cancer
HOEDH76	Y93912	WO200039166-A1	Cancer
HOGCC45	Y93951	WO200039136-A2	Cancer
HTSGS30	Y93973	WO200042189-A1	Digestive, Immune/Hematopoietic, Mixed Fetal
HTSGS30	Y93975	WO200042189-A1	Digestive, Immune/Hematopoietic, Mixed Fetal
HMWCF06	Y94802	WO200009148-A1	Cancer
HE9DR66	Y95534	WO200040726-A1	Cancer
HGBAN46	Y95535	WO200040726-A1	Cancer
HE9DR66	Y95563	WO200040726-A1	Cancer
HE9DR66	Y95565	WO200040726-A1	Cancer
HE9DR66	Y95566	WO200040726-A1	Cancer
HE9DR66	Y95567	WO200040726-A1	Cancer
HE9DR66	Y95568	WO200040726-A1	Cancer
HE9DR66	Y95569	WO200040726-A1	Cancer

HE9DR66	Y95570	WO200040726-A1	Cancer
HE9DR66	Y95571	WO200040726-A1	Cancer
HE9DR66	Y95572	WO200040726-A1	Cancer
HE9DR66	Y95573	WO200040726-A1	Cancer
HE9DR66	Y95574	WO200040726-A1	Cancer
HE9DR66	Y95575	WO200040726-A1	Cancer
HE9DR66	Y95576	WO200040726-A1	Cancer
HE9DR66	Y95577	WO200040726-A1	Cancer
HE9DR66	Y95578	WO200040726-A1	Cancer
HHEAC71	Y95879	WO200050459-A1	Connective/Epithelial, Immune/Hematopoietic
HCEAZ22	Y95880	WO200050459-A1	Cancer
HT5EA78	Y95881	WO200050459-A1	Connective/Epithelial, Immune/Hematopoietic
HDPK85	Y96099	WO200052135-A2	Cancer
HWLLM34	Y96100	WO200052135-A2	Cancer
HA5AA37	Y96101	WO200052135-A2	Cancer
HAPAT57	Y96280	WO200028035-A1	Cancer
HAPAT57	Y96282	WO200028035-A1	Cancer
HKABZ65	Y96962	WO200039327-A1	Connective/Epithelial
HWHGB15	Y96963	WO200039327-A1	Connective/Epithelial
HCDDP40	Y96964	WO200039327-A1	Immune/Hematopoietic, Musculoskeletal
HOSBD47	Y97144	WO200045835-A1	Cancer
HOSBD47	Y97145	WO200045835-A1	Cancer
HFITF82	SEQ ID NO:73		Immune/Hematopoietic, Musculoskeletal
HFITF82	SEQ ID NO:74		Immune/Hematopoietic, Musculoskeletal
HFITF82	SEQ ID NO:75		Immune/Hematopoietic, Musculoskeletal
HFITF82	SEQ ID NO:76		Immune/Hematopoietic, Musculoskeletal
HBZAI19	SEQ ID NO:77		Immune/Hematopoietic, Reproductive
HBZAI19	SEQ ID NO:78		Immune/Hematopoietic, Reproductive
HBZAI19	SEQ ID NO:79		Immune/Hematopoietic, Reproductive
HDPDI45	SEQ ID NO:80		Cancer
HDPDI45	SEQ ID NO:81		Cancer
HETHW90	SEQ ID NO:82		Cancer
HETHW90	SEQ ID NO:83		Cancer
HETHW90	SEQ ID NO:84		Cancer
HIBEB47	SEQ ID NO:85		Digestive, Mixed Fetal, Neural/Sensory
HIBEB47	SEQ ID NO:86		Digestive, Mixed Fetal, Neural/Sensory
HIBEB47	SEQ ID NO:87		Digestive, Mixed Fetal, Neural/Sensory
HIBEB47	SEQ ID NO:88		Digestive, Mixed Fetal, Neural/Sensory

HLHFR58	SEQ ID NO:89		Cancer
HLHFR58	SEQ ID NO:90		Cancer
HLHFR58	SEQ ID NO:91		Cancer
HLHFR58	SEQ ID NO:92		Cancer
HNGGK54	SEQ ID NO:93		Cancer
HNGGK54	SEQ ID NO:94		Cancer
HNGGK54	SEQ ID NO:95		Cancer
HNGGK54	SEQ ID NO:96		Cancer
HUSIE23	SEQ ID NO:97		Cancer
HUSIE23	SEQ ID NO:98		Cancer
HARMB79	SEQ ID NO:99		Cancer
HARMB79	SEQ ID NO:100		Cancer
HJBCY84	SEQ ID NO:101		Cancer
HJBCY84	SEQ ID NO:102		Cancer
HJBCY84	SEQ ID NO:103		Cancer
HCMSC92	SEQ ID NO:104		Cancer
HCMSC92	SEQ ID NO:105		Cancer
HE2AX96	SEQ ID NO:106		Mixed Fetal
HE2AX96	SEQ ID NO:107		Mixed Fetal
HE2AX96	SEQ ID NO:108		Mixed Fetal
HHPDV90	SEQ ID NO:109		Cancer
HHPDV90	SEQ ID NO:110		Cancer
HHPDV90	SEQ ID NO:111		Cancer
HT2SG64	SEQ ID NO:112		Digestive, Immune/Hematopoietic
HT2SG64	SEQ ID NO:113		Digestive, Immune/Hematopoietic
HT2SG64	SEQ ID NO:114		Digestive, Immune/Hematopoietic
HAGAN21	SEQ ID NO:115		Digestive, Immune/Hematopoietic, Neural/Sensory
HAGAN21	SEQ ID NO:116		Digestive, Immune/Hematopoietic, Neural/Sensory
HAGAN21	SEQ ID NO:117		Digestive, Immune/Hematopoietic, Neural/Sensory
HAGAN21	SEQ ID NO:118		Digestive, Immune/Hematopoietic, Neural/Sensory
HAGAN21	SEQ ID NO:119		Digestive, Immune/Hematopoietic, Neural/Sensory
HEBAH57	SEQ ID NO:120		Neural/Sensory
HEBAH57	SEQ ID NO:121		Neural/Sensory
HEBAH57	SEQ ID NO:122		Neural/Sensory
HETDB76	SEQ ID NO:123		Musculoskeletal, Reproductive
HETDB76	SEQ ID NO:124		Musculoskeletal, Reproductive
HETDB76	SEQ ID NO:125		Musculoskeletal, Reproductive
HETDB76	SEQ ID NO:126		Musculoskeletal, Reproductive
HE8SE91	SEQ ID NO:127		Cancer

HE8SE91	SEQ ID NO:128		Cancer
HE8SE91	SEQ ID NO:129		Cancer
HRGBL78	SEQ ID NO:130		Cancer
HRGBL78	SEQ ID NO:131		Cancer
HRGBL78	SEQ ID NO:132		Cancer
HRGBL78	SEQ ID NO:133		Cancer
HHFUC40	SEQ ID NO:134		Cardiovascular
HHFUC40	SEQ ID NO:135		Cardiovascular
HETCP58	SEQ ID NO:136		Immune/Hematopoietic, Reproductive
HETCP58	SEQ ID NO:137		Immune/Hematopoietic, Reproductive
HETCP58	SEQ ID NO:138		Immune/Hematopoietic, Reproductive
HTTBM40	SEQ ID NO:139		Cancer
HTTBM40	SEQ ID NO:140		Cancer
HTTBS64	SEQ ID NO:141		Reproductive
HTTBS64	SEQ ID NO:142		Reproductive
HTTBS64	SEQ ID NO:143		Reproductive
HCEVB32	SEQ ID NO:144		Cancer
HCEVB32	SEQ ID NO:145		Cancer
HCEVB32	SEQ ID NO:146		Cancer
HCEVB32	SEQ ID NO:147		Cancer
HHPFU18	SEQ ID NO:148		Cancer
HHPFU18	SEQ ID NO:149		Cancer
HPRCA90	SEQ ID NO:150		Cancer
HPRCA90	SEQ ID NO:151		Cancer
HPRCA90	SEQ ID NO:152		Cancer
HPRCA90	SEQ ID NO:153		Cancer
HPRCE33	SEQ ID NO:154		Cancer
HPRCE33	SEQ ID NO:155		Cancer
HHFFU55	SEQ ID NO:156		Cardiovascular, Immune/Hematopoietic
HHFFU55	SEQ ID NO:157		Cardiovascular, Immune/Hematopoietic
HUVPD63	SEQ ID NO:158		Cancer
HUVPD63	SEQ ID NO:159		Cancer
HUVPD63	SEQ ID NO:160		Cancer
HUVPD63	SEQ ID NO:161		Cancer
HUVPD63	SEQ ID NO:162		Cancer
HCEFI77	SEQ ID NO:163		Neural/Sensory
HCEFI77	SEQ ID NO:164		Neural/Sensory
HCEFI77	SEQ ID NO:165		Neural/Sensory
HHFDH56	SEQ ID NO:166		Cancer
HHFDN48	SEQ ID NO:167		Cancer
HHFDN48	SEQ ID NO:168		Cancer
HHFDN48	SEQ ID NO:169		Cancer
HHFDN48	SEQ ID NO:170		Cancer
HHFDN48	SEQ ID NO:171		Cancer
HHFDN67	SEQ ID NO:172		Cardiovascular
HHFDN67	SEQ ID NO:173		Cardiovascular
HHFDG51	SEQ ID NO:174		Connective/Epithelial, Musculoskeletal
HHFDG51	SEQ ID NO:175		Connective/Epithelial, Musculoskeletal
HHFDG51	SEQ ID NO:176		Connective/Epithelial,

			Musculoskeletal
HE8AO36	SEQ ID NO:177		Cancer
HE8AO36	SEQ ID NO:178		Cancer
HE8AO36	SEQ ID NO:179		Cancer
HTPAB57	SEQ ID NO:180		Cancer
HTPAB57	SEQ ID NO:181		Cancer
HTPAB57	SEQ ID NO:182		Cancer
HTPAB57	SEQ ID NO:183		Cancer
HFXAX45	SEQ ID NO:184		Neural/Sensory
HFXAX45	SEQ ID NO:185		Neural/Sensory
HFXAX45	SEQ ID NO:186		Neural/Sensory
HTLBE23	SEQ ID NO:187		Reproductive
HTLBE23	SEQ ID NO:188		Reproductive
HCQAM33	SEQ ID NO:189		Musculoskeletal, Reproductive
HCQAM33	SEQ ID NO:190		Musculoskeletal, Reproductive
HCQAM33	SEQ ID NO:191		Musculoskeletal, Reproductive
HCEWE17	SEQ ID NO:192		Digestive, Neural/Sensory
HCEWE17	SEQ ID NO:193		Digestive, Neural/Sensory
HCEWE17	SEQ ID NO:194		Digestive, Neural/Sensory
HTEGI42	SEQ ID NO:195		Cancer
HTEGI42	SEQ ID NO:196		Cancer
HTEGI42	SEQ ID NO:197		Cancer
HTEGI42	SEQ ID NO:198		Cancer
HTEGI42	SEQ ID NO:199		Cancer
HCEIE80	SEQ ID NO:200		Cancer
HCEIE80	SEQ ID NO:201		Cancer
HCEIE80	SEQ ID NO:202		Cancer
HCEIE80	SEQ ID NO:203		Cancer
HLMCA92	SEQ ID NO:204		Digestive, Immune/Hematopoietic, Neural/Sensory
HLMCA92	SEQ ID NO:205		Digestive, Immune/Hematopoietic, Neural/Sensory
HLMCA92	SEQ ID NO:206		Digestive, Immune/Hematopoietic, Neural/Sensory
HLMCA92	SEQ ID NO:207		Digestive, Immune/Hematopoietic, Neural/Sensory
HLHCF36	SEQ ID NO:208		Respiratory
HLHCF36	SEQ ID NO:209		Respiratory
HLHCF36	SEQ ID NO:210		Respiratory
HCEZR26	SEQ ID NO:211		Cancer
HCEZR26	SEQ ID NO:212		Cancer
HGBCO51	SEQ ID NO:213		Cancer
HGBCO51	SEQ ID NO:214		Cancer
HGBCO51	SEQ ID NO:215		Cancer
HGBCO51	SEQ ID NO:216		Cancer
HTABP30	SEQ ID NO:217		Cancer



HTABP30	SEQ ID NO:218		Cancer
HUKCD10	SEQ ID NO:219		Cancer
HUKCD10	SEQ ID NO:220		Cancer
HUKCD10	SEQ ID NO:221		Cancer
HOUHT39	SEQ ID NO:222		Cancer
HOUHT39	SEQ ID NO:223		Cancer
HOUHT39	SEQ ID NO:224		Cancer
HTXBN56	SEQ ID NO:225		Cancer
HTXBN56	SEQ ID NO:226		Cancer
HTXBN56	SEQ ID NO:227		Cancer
HETEU28	SEQ ID NO:228		Cancer
HETEU28	SEQ ID NO:229		Cancer
HODDD43	SEQ ID NO:230		Cancer
HODDD43	SEQ ID NO:231		Cancer
HODDD43	SEQ ID NO:232		Cancer
HPWAL61	SEQ ID NO:233		Musculoskeletal, Reproductive
HPWAL61	SEQ ID NO:234		Musculoskeletal, Reproductive
HPWAL61	SEQ ID NO:235		Musculoskeletal, Reproductive
HPWAL61	SEQ ID NO:236		Musculoskeletal, Reproductive
HTSER67	SEQ ID NO:237		Cancer
HTSER67	SEQ ID NO:238		Cancer
HMSDL37	SEQ ID NO:239		Cancer
HMSDL37	SEQ ID NO:240		Cancer
HMSDL37	SEQ ID NO:241		Cancer
HMSDL37	SEQ ID NO:242		Cancer
HSDAJ53	SEQ ID NO:243		Cancer
HSDAJ53	SEQ ID NO:244		Cancer
HSDAJ53	SEQ ID NO:245		Cancer
HSDAJ53	SEQ ID NO:246		Cancer
HEBDF05	SEQ ID NO:247		Neural/Sensory
HEBDF05	SEQ ID NO:248		Neural/Sensory
HEBDF05	SEQ ID NO:249		Neural/Sensory
HSQFT30	SEQ ID NO:250		Cancer
HSQFT30	SEQ ID NO:251		Cancer
HSIDX71	SEQ ID NO:252		Digestive, Neural/Sensory
HSIDX71	SEQ ID NO:253		Digestive, Neural/Sensory
HSAUA82	SEQ ID NO:254		Immune/Hematopoietic, Reproductive
HSAUA82	SEQ ID NO:255		Immune/Hematopoietic, Reproductive
HPWAY46	SEQ ID NO:256		Cancer
HPWAY46	SEQ ID NO:257		Cancer
HPWAY46	SEQ ID NO:258		Cancer
HSEN70	SEQ ID NO:259		Cancer
HSEN70	SEQ ID NO:260		Cancer
HTOHB55	SEQ ID NO:261		Cancer
HTOHB55	SEQ ID NO:262		Cancer
HTOHM15	SEQ ID NO:263		Cancer
HTOHM15	SEQ ID NO:264		Cancer
HTOHM15	SEQ ID NO:265		Cancer

HTOHM15	SEQ ID NO:266	Cancer
HHNAB56	SEQ ID NO:267	Digestive
HHNAB56	SEQ ID NO:268	Digestive
HHNAB56	SEQ ID NO:269	Digestive
HJABL02	SEQ ID NO:270	Cancer
HJABL02	SEQ ID NO:271	Cancer
HJACG30	SEQ ID NO:272	Immune/Hematopoietic
HJACG30	SEQ ID NO:273	Immune/Hematopoietic
HJACG30	SEQ ID NO:274	Immune/Hematopoietic
HTAEE28	SEQ ID NO:275	Digestive, Immune/Hematopoietic, Mixed Fetal
HTAEE28	SEQ ID NO:276	Digestive, Immune/Hematopoietic, Mixed Fetal
HTAEE28	SEQ ID NO:277	Digestive, Immune/Hematopoietic, Mixed Fetal
HTHBG43	SEQ ID NO:278	Immune/Hematopoietic
HTHBG43	SEQ ID NO:279	Immune/Hematopoietic
HJPCE80	SEQ ID NO:280	Cancer
HJPCE80	SEQ ID NO:281	Cancer
HJPCE80	SEQ ID NO:282	Cancer
HTOIZ02	SEQ ID NO:283	Cancer
HTOIZ02	SEQ ID NO:284	Cancer
HJPCR70	SEQ ID NO:285	Cancer
HJPCR70	SEQ ID NO:286	Cancer
HJPCR70	SEQ ID NO:287	Cancer
HJPCR70	SEQ ID NO:288	Cancer
HJPCP42	SEQ ID NO:289	Digestive, Immune/Hematopoietic
HJPCP42	SEQ ID NO:290	Digestive, Immune/Hematopoietic
HJPCP42	SEQ ID NO:291	Digestive, Immune/Hematopoietic
HJPCP42	SEQ ID NO:292	Digestive, Immune/Hematopoietic
HNFFD47	SEQ ID NO:293	Immune/Hematopoietic
HNFFD47	SEQ ID NO:294	Immune/Hematopoietic
HNFFD47	SEQ ID NO:295	Immune/Hematopoietic
HNFFI46	SEQ ID NO:296	Cancer
HNFFI46	SEQ ID NO:297	Cancer
HNFFI46	SEQ ID NO:298	Cancer
HNFFI46	SEQ ID NO:299	Cancer
HNFFI46	SEQ ID NO:300	Cancer
HTOIQ42	SEQ ID NO:301	Cancer
HTOIQ42	SEQ ID NO:302	Cancer
HLTDW13	SEQ ID NO:303	Cancer
HLTDW13	SEQ ID NO:304	Cancer
HLTDW13	SEQ ID NO:305	Cancer
HLTDW13	SEQ ID NO:306	Cancer
HLTDW13	SEQ ID NO:307	Cancer
HLTDY51	SEQ ID NO:308	Cancer
HLTDY51	SEQ ID NO:309	Cancer
HNFFZ56	SEQ ID NO:310	Cancer
HNFFZ56	SEQ ID NO:311	Cancer

HNGAV54	SEQ ID NO:312		Immune/Hematopoietic
HNGAV54	SEQ ID NO:313		Immune/Hematopoietic
HSLCA15	SEQ ID NO:314		Cancer
HSLCA15	SEQ ID NO:315		Cancer
HSLCA15	SEQ ID NO:316		Cancer
HSLCA15	SEQ ID NO:317		Cancer
HSLCA15	SEQ ID NO:318		Cancer
HSLCA15	SEQ ID NO:319		Cancer
HSLCP57	SEQ ID NO:320		Cancer
HSLCP57	SEQ ID NO:321		Cancer
HTOJP95	SEQ ID NO:322		Immune/Hematopoietic
HTOJP95	SEQ ID NO:323		Immune/Hematopoietic
HBMVI55	SEQ ID NO:324		Cancer
HBMVI55	SEQ ID NO:325		Cancer
HBMVI55	SEQ ID NO:326		Cancer
HBMVI55	SEQ ID NO:327		Cancer
HBMVI55	SEQ ID NO:328		Cancer
HFXBS68	SEQ ID NO:329		Neural/Sensory
HFXBS68	SEQ ID NO:330		Neural/Sensory
HFXBS68	SEQ ID NO:331		Neural/Sensory
HFXBS68	SEQ ID NO:332		Neural/Sensory
HNGBC07	SEQ ID NO:333		Immune/Hematopoietic
HNGBC07	SEQ ID NO:334		Immune/Hematopoietic
HNGBC07	SEQ ID NO:335		Immune/Hematopoietic
HMSFK67	SEQ ID NO:336		Cancer
HMSFK67	SEQ ID NO:337		Cancer
HMSFK67	SEQ ID NO:338		Cancer
HCE1P80	SEQ ID NO:339		Cancer
HCE1P80	SEQ ID NO:340		Cancer
HCE1P80	SEQ ID NO:341		Cancer
HOUDU29	SEQ ID NO:342		Cancer
HOUDU29	SEQ ID NO:343		Cancer
HOUDU29	SEQ ID NO:344		Cancer
HOUDU29	SEQ ID NO:345		Cancer
HOUDU29	SEQ ID NO:346		Cancer
HHFEC49	SEQ ID NO:347		Cancer
HCE3T57	SEQ ID NO:348		Immune/Hematopoietic, Neural/Sensory, Reproductive
HCE3T57	SEQ ID NO:349		Immune/Hematopoietic, Neural/Sensory, Reproductive
HCE3T57	SEQ ID NO:350		Immune/Hematopoietic, Neural/Sensory, Reproductive
HCE3T57	SEQ ID NO:351		Immune/Hematopoietic, Neural/Sensory, Reproductive
HCE3T57	SEQ ID NO:352		Immune/Hematopoietic, Neural/Sensory, Reproductive
HCE4Y07	SEQ ID NO:353		Cancer
HCE4Y07	SEQ ID NO:354		Cancer
HCE5G23	SEQ ID NO:355		Cancer
HCE5G23	SEQ ID NO:356		Cancer
HCE5G23	SEQ ID NO:357		Cancer

HFCEP45	SEQ ID NO:358		Neural/Sensory
HFCEP45	SEQ ID NO:359		Neural/Sensory
HFCEP45	SEQ ID NO:360		Neural/Sensory
HFCEP45	SEQ ID NO:361		Neural/Sensory
HMWEJ52	SEQ ID NO:362		Immune/Hematopoietic
HMWEJ52	SEQ ID NO:363		Immune/Hematopoietic
HMWEY26	SEQ ID NO:364		Cancer
HMWEY26	SEQ ID NO:365		Cancer
HMWEY26	SEQ ID NO:366		Cancer
HMWEY26	SEQ ID NO:367		Cancer
HMWEY26	SEQ ID NO:368		Cancer
HATDM46	SEQ ID NO:369		Cancer
HATDM46	SEQ ID NO:370		Cancer
HATDM46	SEQ ID NO:371		Cancer
HATDM46	SEQ ID NO:372		Cancer
HATDM46	SEQ ID NO:373		Cancer
HATDM46	SEQ ID NO:374		Cancer
HHFHD37	SEQ ID NO:375		Cardiovascular, Immune/Hematopoietic, Respiratory
HHFHD37	SEQ ID NO:376		Cardiovascular, Immune/Hematopoietic, Respiratory
HHFHI76	SEQ ID NO:377		Cancer
HHFHI76	SEQ ID NO:378		Cancer
HATDZ29	SEQ ID NO:379		Endocrine, Immune/Hematopoietic
HATDZ29	SEQ ID NO:380		Endocrine, Immune/Hematopoietic
HFVGE32	SEQ ID NO:381		Digestive, Immune/Hematopoietic
HFVGE32	SEQ ID NO:382		Digestive, Immune/Hematopoietic
HLHFE92	SEQ ID NO:383		Cancer
HLHFE92	SEQ ID NO:384		Cancer
HLHFE92	SEQ ID NO:385		Cancer
HMKAI25	SEQ ID NO:386		Cancer
HMKAI25	SEQ ID NO:387		Cancer
HMKAI25	SEQ ID NO:388		Cancer
HMKAI25	SEQ ID NO:389		Cancer
HMKAI25	SEQ ID NO:390		Cancer
HNHEI42	SEQ ID NO:391		Endocrine, Immune/Hematopoietic
HNHEI42	SEQ ID NO:392		Endocrine, Immune/Hematopoietic
HNHEI42	SEQ ID NO:393		Endocrine, Immune/Hematopoietic
HNHEI42	SEQ ID NO:394		Endocrine, Immune/Hematopoietic
HNHEI85	SEQ ID NO:395		Digestive, Immune/Hematopoietic, Musculoskeletal
HNHEI85	SEQ ID NO:396		Digestive, Immune/Hematopoietic, Musculoskeletal
HOEDE28	SEQ ID NO:397		Cancer

HOEDE28	SEQ ID NO:398		Cancer
H2CBH03	SEQ ID NO:399		Cancer
HTHCA18	SEQ ID NO:400		Immune/Hematopoietic
HTHCA18	SEQ ID NO:401		Immune/Hematopoietic
HTHCO79	SEQ ID NO:402		Cancer
HTHCO79	SEQ ID NO:403		Cancer
HNGFB76	SEQ ID NO:404		Digestive, Immune/Hematopoietic, Neural/Sensory
HNGFB76	SEQ ID NO:405		Digestive, Immune/Hematopoietic, Neural/Sensory
HNGFB76	SEQ ID NO:406		Digestive, Immune/Hematopoietic, Neural/Sensory
HNGFB76	SEQ ID NO:407		Digestive, Immune/Hematopoietic, Neural/Sensory
HOQBJ82	SEQ ID NO:408		Cancer
HOQBJ82	SEQ ID NO:409		Cancer
HNFBHY51	SEQ ID NO:410		Immune/Hematopoietic, Reproductive
HNFBHY51	SEQ ID NO:411		Immune/Hematopoietic, Reproductive
HNFBHY51	SEQ ID NO:412		Immune/Hematopoietic, Reproductive
HNFBHY51	SEQ ID NO:413		Immune/Hematopoietic, Reproductive
HNEEB45	SEQ ID NO:414		Immune/Hematopoietic, Mixed Fetal
HNEEB45	SEQ ID NO:415		Immune/Hematopoietic, Mixed Fetal
HSDFA44	SEQ ID NO:416		Neural/Sensory
HSDFA44	SEQ ID NO:417		Neural/Sensory
HSDFA44	SEQ ID NO:418		Neural/Sensory
HAGEB14	SEQ ID NO:419		Cancer
HAGEB14	SEQ ID NO:420		Cancer
HCGBE81	SEQ ID NO:421		Neural/Sensory, Reproductive
HCGBE81	SEQ ID NO:422		Neural/Sensory, Reproductive
HEOMX53	SEQ ID NO:423		Digestive, Immune/Hematopoietic, Neural/Sensory
HEOMX53	SEQ ID NO:424		Digestive, Immune/Hematopoietic, Neural/Sensory
HEOMX53	SEQ ID NO:425		Digestive, Immune/Hematopoietic, Neural/Sensory
HEONC95	SEQ ID NO:426		Cancer
HEONC95	SEQ ID NO:427		Cancer
HKMLP68	SEQ ID NO:428		Excretory, Mixed Fetal, Reproductive
HKMLP68	SEQ ID NO:429		Excretory,

			Mixed Fetal, Reproductive
HKMLP68	SEQ ID NO:430		Excretory, Mixed Fetal, Reproductive
HMWIG83	SEQ ID NO:431		Cancer
HMWIG83	SEQ ID NO:432		Cancer
HMSKH19	SEQ ID NO:433		Cancer
HMSKH19	SEQ ID NO:434		Cancer
HMSKH19	SEQ ID NO:435		Cancer
HFAME37	SEQ ID NO:436		Neural/Sensory
HFAME37	SEQ ID NO:437		Neural/Sensory
HFAME37	SEQ ID NO:438		Neural/Sensory
HFXFG45	SEQ ID NO:439		Immune/Hematopoietic, Neural/Sensory
HFXFG45	SEQ ID NO:440		Immune/Hematopoietic, Neural/Sensory
HFXFG45	SEQ ID NO:441		Immune/Hematopoietic, Neural/Sensory
HFXFG45	SEQ ID NO:442		Immune/Hematopoietic, Neural/Sensory
HFXFH04	SEQ ID NO:443		Immune/Hematopoietic, Neural/Sensory
HFXFH04	SEQ ID NO:444		Immune/Hematopoietic, Neural/Sensory
HFXFH04	SEQ ID NO:445		Immune/Hematopoietic, Neural/Sensory
HFXFH04	SEQ ID NO:446		Immune/Hematopoietic, Neural/Sensory
HGCAC66	SEQ ID NO:447		Cancer
HGCAC66	SEQ ID NO:448		Cancer
HSSJF55	SEQ ID NO:449		Musculoskeletal
HSSJF55	SEQ ID NO:450		Musculoskeletal
HFXHM17	SEQ ID NO:451		Cancer
HFXHM17	SEQ ID NO:452		Cancer
HFXHM17	SEQ ID NO:453		Cancer
HFXHM17	SEQ ID NO:454		Cancer
HOSFQ65	SEQ ID NO:455		Cancer
HOSFQ65	SEQ ID NO:456		Cancer
HOSFQ65	SEQ ID NO:457		Cancer
HOSFQ65	SEQ ID NO:458		Cancer
HOSFQ65	SEQ ID NO:459		Cancer
HKGAS32	SEQ ID NO:460		Connective/Epithelial, Neural/Sensory
HKGAS32	SEQ ID NO:461		Connective/Epithelial, Neural/Sensory
HKGAU45	SEQ ID NO:462		Immune/Hematopoietic
HKGAU45	SEQ ID NO:463		Immune/Hematopoietic
HKGAU45	SEQ ID NO:464		Immune/Hematopoietic
HKGBH24	SEQ ID NO:465		Cancer
HKGBH24	SEQ ID NO:466		Cancer
HKGBH24	SEQ ID NO:467		Cancer
HKGBS01	SEQ ID NO:468		Cancer
HKGBS01	SEQ ID NO:469		Cancer
HKGBS01	SEQ ID NO:470		Cancer
HACCL63	SEQ ID NO:471		Cancer

HACCL63	SEQ ID NO:472		Cancer
HACCL63	SEQ ID NO:473		Cancer
HACCL63	SEQ ID NO:474		Cancer
HFIIN69	SEQ ID NO:475		Musculoskeletal, Neural/Sensory, Reproductive
HFIIN69	SEQ ID NO:476		Musculoskeletal, Neural/Sensory, Reproductive
HFIIN69	SEQ ID NO:477		Musculoskeletal, Neural/Sensory, Reproductive
HFILZ70	SEQ ID NO:478		Cancer
HFILZ70	SEQ ID NO:479		Cancer
HMLAJ30	SEQ ID NO:480		Cancer
HMLAJ30	SEQ ID NO:481		Cancer
HMLAJ30	SEQ ID NO:482		Cancer
HMLAJ30	SEQ ID NO:483		Cancer
HMLAV73	SEQ ID NO:484		Cancer
HMLAV73	SEQ ID NO:485		Cancer
HMLAV73	SEQ ID NO:486		Cancer
HMLAV73	SEQ ID NO:487		Cancer
HAPOD80	SEQ ID NO:488		Cancer
HISBL03	SEQ ID NO:489		Cancer
HISBL03	SEQ ID NO:490		Cancer
HISBL03	SEQ ID NO:491		Cancer
HISBL03	SEQ ID NO:492		Cancer
HISBL03	SEQ ID NO:493		Cancer
HISBL03	SEQ ID NO:494		Cancer
HISBL03	SEQ ID NO:495		Cancer
HMICK94	SEQ ID NO:496		Cancer
HMICK94	SEQ ID NO:497		Cancer
HMICK94	SEQ ID NO:498		Cancer
HISBF60	SEQ ID NO:499		Cancer
HISBF60	SEQ ID NO:500		Cancer
HISBF60	SEQ ID NO:501		Cancer
HISBF60	SEQ ID NO:502		Cancer
HISBF60	SEQ ID NO:503		Cancer
HMVAV54	SEQ ID NO:504		Immune/Hematopoietic
HMVAV54	SEQ ID NO:505		Immune/Hematopoietic
HMVAV54	SEQ ID NO:506		Immune/Hematopoietic
HMVAV54	SEQ ID NO:507		Immune/Hematopoietic
HMVAV54	SEQ ID NO:508		Immune/Hematopoietic
HPICB53	SEQ ID NO:509		Cancer
HPICB53	SEQ ID NO:510		Cancer
HPICC86	SEQ ID NO:511		Reproductive
HPICC86	SEQ ID NO:512		Reproductive
HPICC86	SEQ ID NO:513		Reproductive
HPICC86	SEQ ID NO:514		Reproductive
HPICC86	SEQ ID NO:515		Reproductive
HPJAP43	SEQ ID NO:516		Cancer
HPJAP43	SEQ ID NO:517		Cancer
HPJAP43	SEQ ID NO:518		Cancer
HPJCG42	SEQ ID NO:519		Immune/Hematopoietic, Reproductive
HPJCG42	SEQ ID NO:520		Immune/Hematopoietic,

			Reproductive
HPJCG42	SEQ ID NO:521		Immune/Hematopoietic, Reproductive
HPJCG42	SEQ ID NO:522		Immune/Hematopoietic, Reproductive
HPJCG42	SEQ ID NO:523		Immune/Hematopoietic, Reproductive
HPJBK11	SEQ ID NO:524		Cardiovascular, Neural/Sensory, Reproductive
HPJBK11	SEQ ID NO:525		Cardiovascular, Neural/Sensory, Reproductive
HPJBK11	SEQ ID NO:526		Cardiovascular, Neural/Sensory, Reproductive
HPJBK12	SEQ ID NO:527		Reproductive
HPJBK12	SEQ ID NO:528		Reproductive
HPJBK12	SEQ ID NO:529		Reproductive
HPJBK12	SEQ ID NO:530		Reproductive
HPJCT08	SEQ ID NO:531		Connective/Epithelial, Reproductive
HPJCT08	SEQ ID NO:532		Connective/Epithelial, Reproductive
HPJCT08	SEQ ID NO:533		Connective/Epithelial, Reproductive
HT4ES80	SEQ ID NO:534		Cancer
HT4ES80	SEQ ID NO:535		Cancer
HT4ES80	SEQ ID NO:536		Cancer
HNTNB49	SEQ ID NO:537		Cancer
HNTNB49	SEQ ID NO:538		Cancer
HNTRS57	SEQ ID NO:539		Cancer
HNTRS57	SEQ ID NO:540		Cancer
HNTRS57	SEQ ID NO:541		Cancer
HNTRS57	SEQ ID NO:542		Cancer
HNTRS57	SEQ ID NO:543		Cancer
HNTSL47	SEQ ID NO:544		Cardiovascular, Digestive
HNTSL47	SEQ ID NO:545		Cardiovascular, Digestive
HNTSL47	SEQ ID NO:546		Cardiovascular, Digestive
HBJLR70	SEQ ID NO:547		Immune/Hematopoietic, Neural/Sensory
HBJLR70	SEQ ID NO:548		Immune/Hematopoietic, Neural/Sensory
HNTSY18	SEQ ID NO:549		Cardiovascular, Reproductive
HNTSY18	SEQ ID NO:550		Cardiovascular, Reproductive
HBHME51	SEQ ID NO:551		Reproductive, Respiratory
HBHME51	SEQ ID NO:552		Reproductive, Respiratory
HBHME51	SEQ ID NO:553		Reproductive, Respiratory



HMCHR48	SEQ ID NO:554		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HMCHR48	SEQ ID NO:555		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HMCHR48	SEQ ID NO:556		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HMCIJ07	SEQ ID NO:557		Immune/Hematopoietic
HMCIJ07	SEQ ID NO:558		Immune/Hematopoietic
HSIFL06	SEQ ID NO:559		Cancer
HSIFL06	SEQ ID NO:560		Cancer
HMZME33	SEQ ID NO:561		Connective/Epithelial, Digestive
HMZME33	SEQ ID NO:562		Connective/Epithelial, Digestive
HMZMF54	SEQ ID NO:563		Digestive
HMZMF54	SEQ ID NO:564		Digestive
HMZMF54	SEQ ID NO:565		Digestive
HMVCQ82	SEQ ID NO:566		Immune/Hematopoietic
HMVCQ82	SEQ ID NO:567		Immune/Hematopoietic
HMVCQ82	SEQ ID NO:568		Immune/Hematopoietic
HMVDP35	SEQ ID NO:569		Immune/Hematopoietic, Reproductive
HMVDP35	SEQ ID NO:570		Immune/Hematopoietic, Reproductive
HMVDP35	SEQ ID NO:571		Immune/Hematopoietic, Reproductive
HMVDF54	SEQ ID NO:572		Cancer
HMVDF54	SEQ ID NO:573		Cancer
HMVDF54	SEQ ID NO:574		Cancer
HROBM46	SEQ ID NO:575		Connective/Epithelial, Digestive
HROBM46	SEQ ID NO:576		Connective/Epithelial, Digestive
HCNDR47	SEQ ID NO:577		Cancer
HCNDR47	SEQ ID NO:578		Cancer
HCNDR47	SEQ ID NO:579		Cancer
HCNDV12	SEQ ID NO:580		Digestive, Reproductive
HCNDV12	SEQ ID NO:581		Digestive, Reproductive
HCNDV12	SEQ ID NO:582		Digestive, Reproductive
HSODE04	SEQ ID NO:583		Digestive
HSODE04	SEQ ID NO:584		Digestive
HBFMC03	SEQ ID NO:585		Digestive, Musculoskeletal, Reproductive
HBFMC03	SEQ ID NO:586		Digestive, Musculoskeletal, Reproductive
HHSFB67	SEQ ID NO:587		Neural/Sensory
HHSFB67	SEQ ID NO:588		Neural/Sensory
HHSFB67	SEQ ID NO:589		Neural/Sensory

HHSFB67	SEQ ID NO:590	Neural/Sensory
HHSGW69	SEQ ID NO:591	Cancer
HHSGW69	SEQ ID NO:592	Cancer
HHSGW69	SEQ ID NO:593	Cancer
HCLCJ15	SEQ ID NO:594	Cancer
HCLCJ15	SEQ ID NO:595	Cancer
HCLCJ15	SEQ ID NO:596	Cancer
HCLCJ15	SEQ ID NO:597	Cancer
HSLJG37	SEQ ID NO:598	Cancer
HSLJG37	SEQ ID NO:599	Cancer
HSLJG37	SEQ ID NO:600	Cancer
HWLEC41	SEQ ID NO:601	Cancer
HWLEC41	SEQ ID NO:602	Cancer
HWLEC41	SEQ ID NO:603	Cancer
HSXEQ06	SEQ ID NO:604	Cancer
HSXEQ06	SEQ ID NO:605	Cancer
HSXEQ06	SEQ ID NO:606	Cancer
HEEAA16	SEQ ID NO:607	Cancer
HEEAA16	SEQ ID NO:608	Cancer
HEEAA16	SEQ ID NO:609	Cancer
HEEAM62	SEQ ID NO:610	Reproductive
HEEAM62	SEQ ID NO:611	Reproductive
HEEAM62	SEQ ID NO:612	Reproductive
HEEAM62	SEQ ID NO:613	Reproductive
HNHKL90	SEQ ID NO:614	Immune/Hematopoietic
HNHKL90	SEQ ID NO:615	Immune/Hematopoietic
HNHKL90	SEQ ID NO:616	Immune/Hematopoietic
HWLFQ64	SEQ ID NO:617	Digestive
HWLFQ64	SEQ ID NO:618	Digestive
HWLFR02	SEQ ID NO:619	Cancer
HWLFR02	SEQ ID NO:620	Cancer
HWLFR02	SEQ ID NO:621	Cancer
HBKED12	SEQ ID NO:622	Cancer
HBKED12	SEQ ID NO:623	Cancer
HBKED12	SEQ ID NO:624	Cancer
HBKED12	SEQ ID NO:625	Cancer
HBKED12	SEQ ID NO:626	Cancer
HWLFJ10	SEQ ID NO:627	Cancer
HWLFJ10	SEQ ID NO:628	Cancer
HCRNO87	SEQ ID NO:629	Cancer
HCRNO87	SEQ ID NO:630	Cancer
HCRNO87	SEQ ID NO:631	Cancer
HCRNO87	SEQ ID NO:632	Cancer
HWLJX42	SEQ ID NO:633	Cancer
HWLJX42	SEQ ID NO:634	Cancer
HWLJX42	SEQ ID NO:635	Cancer
HSPBY63	SEQ ID NO:636	Digestive
HSPBY63	SEQ ID NO:637	Digestive
HSPBY63	SEQ ID NO:638	Digestive
HAPSO15	SEQ ID NO:639	Cancer
HAPSO15	SEQ ID NO:640	Cancer
HAPSO15	SEQ ID NO:641	Cancer
HE8QG24	SEQ ID NO:642	Mixed Fetal
HE8QG24	SEQ ID NO:643	Mixed Fetal
HE8QG24	SEQ ID NO:644	Mixed Fetal

HE8QV43	SEQ ID NO:645	Cancer
HE8QV43	SEQ ID NO:646	Cancer
HE8QV43	SEQ ID NO:647	Cancer
HE8QV43	SEQ ID NO:648	Cancer
HE9QN39	SEQ ID NO:649	Cancer
HE9QN39	SEQ ID NO:650	Cancer
HE9RO44	SEQ ID NO:651	Immune/Hematopoietic, Mixed Fetal
HE9RO44	SEQ ID NO:652	Immune/Hematopoietic, Mixed Fetal
HE9RO44	SEQ ID NO:653	Immune/Hematopoietic, Mixed Fetal
HE9SE18	SEQ ID NO:654	Digestive, Mixed Fetal
HE9SE18	SEQ ID NO:655	Digestive, Mixed Fetal
HE9SE18	SEQ ID NO:656	Digestive, Mixed Fetal
HISCV60	SEQ ID NO:657	Digestive
HISCV60	SEQ ID NO:658	Digestive
HE8UT25	SEQ ID NO:659	Mixed Fetal
HE8UT25	SEQ ID NO:660	Mixed Fetal
HE8UT25	SEQ ID NO:661	Mixed Fetal
HE8UY36	SEQ ID NO:662	Cancer
HE8UY36	SEQ ID NO:663	Cancer
HNHNT13	SEQ ID NO:664	Immune/Hematopoietic
HNHNT13	SEQ ID NO:665	Immune/Hematopoietic
HNHNT13	SEQ ID NO:666	Immune/Hematopoietic
HODEB50	SEQ ID NO:667	Reproductive
HODEB50	SEQ ID NO:668	Reproductive
HODEB50	SEQ ID NO:669	Reproductive
HNGMJ91	SEQ ID NO:670	Immune/Hematopoietic
HNGMJ91	SEQ ID NO:671	Immune/Hematopoietic
HNGMJ91	SEQ ID NO:672	Immune/Hematopoietic
HNGNB69	SEQ ID NO:673	Immune/Hematopoietic
HODFW41	SEQ ID NO:674	Reproductive
HODFW41	SEQ ID NO:675	Reproductive
HNGOI12	SEQ ID NO:676	Immune/Hematopoietic
HNGOI12	SEQ ID NO:677	Immune/Hematopoietic
HNGOI12	SEQ ID NO:678	Immune/Hematopoietic
HNGPM78	SEQ ID NO:679	Immune/Hematopoietic, Neural/Sensory
HNGPM78	SEQ ID NO:680	Immune/Hematopoietic, Neural/Sensory
HYASC80	SEQ ID NO:681	Cancer
HYASC80	SEQ ID NO:682	Cancer
HWLHM66	SEQ ID NO:683	Cancer
HWLHM66	SEQ ID NO:684	Cancer
HWLHM66	SEQ ID NO:685	Cancer
HWLHM66	SEQ ID NO:686	Cancer
HBBBC71	SEQ ID NO:687	Cancer
HBBBC71	SEQ ID NO:688	Cancer
HBBBC71	SEQ ID NO:689	Cancer
HLJBF86	SEQ ID NO:690	Cancer
HLJBF86	SEQ ID NO:691	Cancer
HLJBF86	SEQ ID NO:692	Cancer



HDPML23	SEQ ID NO:745		Immune/Hematopoietic, Neural/Sensory
HDPML23	SEQ ID NO:746		Immune/Hematopoietic, Neural/Sensory
HDPMM88	SEQ ID NO:747		Cancer
HDPMM88	SEQ ID NO:748		Cancer
HDPMM88	SEQ ID NO:749		Cancer
HDPMM88	SEQ ID NO:750		Cancer
HDPMM88	SEQ ID NO:751		Cancer
HDPMM88	SEQ ID NO:752		Cancer
HDPMM88	SEQ ID NO:753		Cancer
HDPMS12	SEQ ID NO:754		Cancer
HDPMS12	SEQ ID NO:755		Cancer
HDPMS12	SEQ ID NO:756		Cancer
HDPMS12	SEQ ID NO:757		Cancer
HDPMS12	SEQ ID NO:758		Cancer
HDPMS12	SEQ ID NO:759		Cancer
HDPAP35	SEQ ID NO:760		Excretory, Immune/Hematopoietic, Neural/Sensory
HDPAP35	SEQ ID NO:761		Excretory, Immune/Hematopoietic, Neural/Sensory
HDPAP35	SEQ ID NO:762		Excretory, Immune/Hematopoietic, Neural/Sensory
HDPAP35	SEQ ID NO:763		Excretory, Immune/Hematopoietic, Neural/Sensory
HDPAQ55	SEQ ID NO:764		Digestive, Immune/Hematopoietic, Reproductive
HDPAQ55	SEQ ID NO:765		Digestive, Immune/Hematopoietic, Reproductive
HDPAQ55	SEQ ID NO:766		Digestive, Immune/Hematopoietic, Reproductive
HDPAQ55	SEQ ID NO:767		Digestive, Immune/Hematopoietic, Reproductive
HKAAV61	SEQ ID NO:768		Connective/Epithelial
HKAAV61	SEQ ID NO:769		Connective/Epithelial
HKAAV61	SEQ ID NO:770		Connective/Epithelial
HDPCJ43	SEQ ID NO:771		Cancer
HDPCJ43	SEQ ID NO:772		Cancer
HDPCJ43	SEQ ID NO:773		Cancer
HDPCJ43	SEQ ID NO:774		Cancer
HKACM93	SEQ ID NO:775		Cancer
HKACM93	SEQ ID NO:776		Cancer
HKACM93	SEQ ID NO:777		Cancer
HKACM93	SEQ ID NO:778		Cancer
HKAFT66	SEQ ID NO:779		Connective/Epithelial, Digestive, Immune/Hematopoietic
HKAFT66	SEQ ID NO:780		Connective/Epithelial,

			Digestive, Immune/Hematopoietic
HKAFT66	SEQ ID NO:781		Connective/Epithelial, Digestive, Immune/Hematopoietic
HHEMM74	SEQ ID NO:782		Cancer
HHEMM74	SEQ ID NO:783		Cancer
HHEMM74	SEQ ID NO:784		Cancer
HHEMM74	SEQ ID NO:785		Cancer
HAMFC93	SEQ ID NO:786		Cancer
HAMFC93	SEQ ID NO:787		Cancer
HAMFC93	SEQ ID NO:788		Cancer
HSYAZ50	SEQ ID NO:789		Cancer
HSYAZ50	SEQ ID NO:790		Cancer
HSYAZ50	SEQ ID NO:791		Cancer
HSYAZ50	SEQ ID NO:792		Cancer
HLWAX42	SEQ ID NO:793		Cancer
HLWAX42	SEQ ID NO:794		Cancer
HLWAX42	SEQ ID NO:795		Cancer
HLWAZ70	SEQ ID NO:796		Cancer
HLWAZ70	SEQ ID NO:797		Cancer
HLWAZ70	SEQ ID NO:798		Cancer
HLWAZ70	SEQ ID NO:799		Cancer
HLWBG83	SEQ ID NO:800		Cancer
HLWBG83	SEQ ID NO:801		Cancer
HLWBG83	SEQ ID NO:802		Cancer
HLWBG83	SEQ ID NO:803		Cancer
HLWBG83	SEQ ID NO:804		Cancer
HLWBH18	SEQ ID NO:805		Reproductive
HLWBH18	SEQ ID NO:806		Reproductive
HRABS65	SEQ ID NO:807		Cancer
HRABV43	SEQ ID NO:808		Cancer
HRABV43	SEQ ID NO:809		Cancer
HRABV43	SEQ ID NO:810		Cancer
HHEPG23	SEQ ID NO:811		Cancer
HHEPG23	SEQ ID NO:812		Cancer
HHEPG23	SEQ ID NO:813		Cancer
HHEPJ23	SEQ ID NO:814		Cancer
HHEPJ23	SEQ ID NO:815		Cancer
HDPIW06	SEQ ID NO:816		Digestive, Immune/Hematopoietic, Neural/Sensory
HDPIW06	SEQ ID NO:817		Digestive, Immune/Hematopoietic, Neural/Sensory
HDPIW06	SEQ ID NO:818		Digestive, Immune/Hematopoietic, Neural/Sensory
HDPIW06	SEQ ID NO:819		Digestive, Immune/Hematopoietic, Neural/Sensory
HDPIW06	SEQ ID NO:820		Digestive, Immune/Hematopoietic, Neural/Sensory
HDPPA04	SEQ ID NO:821		Cardiovascular, Connective/Epithelial,

			Immune/Hematopoietic
HDPPA04	SEQ ID NO:822		Cardiovascular, Connective/Epithelial, Immune/Hematopoietic
HDPPA04	SEQ ID NO:823		Cardiovascular, Connective/Epithelial, Immune/Hematopoietic
HDPPN86	SEQ ID NO:824		Cancer
HDPPN86	SEQ ID NO:825		Cancer
HDTEK44	SEQ ID NO:826		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HDTEK44	SEQ ID NO:827		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HDTEK44	SEQ ID NO:828		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HDTEK44	SEQ ID NO:829		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HOHBL42	SEQ ID NO:830		Cancer
HOHBL42	SEQ ID NO:831		Cancer
HOHBL42	SEQ ID NO:832		Cancer
HOHBL42	SEQ ID NO:833		Cancer
HOHBP82	SEQ ID NO:834		Musculoskeletal
HOHBP82	SEQ ID NO:835		Musculoskeletal
HOHBP82	SEQ ID NO:836		Musculoskeletal
HOHBP82	SEQ ID NO:837		Musculoskeletal
HOHBY44	SEQ ID NO:838		Cancer
HOHBY44	SEQ ID NO:839		Cancer
HOHBY44	SEQ ID NO:840		Cancer
HWBAD01	SEQ ID NO:841		Immune/Hematopoietic
HWBAD01	SEQ ID NO:842		Immune/Hematopoietic
HWBAD01	SEQ ID NO:843		Immune/Hematopoietic
HOHCJ90	SEQ ID NO:844		Cancer
HOHCJ90	SEQ ID NO:845		Cancer
HWABE12	SEQ ID NO:846		Cancer
HWABE12	SEQ ID NO:847		Cancer
HWABE12	SEQ ID NO:848		Cancer
HWBAR14	SEQ ID NO:849		Cancer
HWBAR14	SEQ ID NO:850		Cancer
HWBAR14	SEQ ID NO:851		Cancer
HWBAR14	SEQ ID NO:852		Cancer
HWBAR88	SEQ ID NO:853		Cancer
HWBCH13	SEQ ID NO:854		Immune/Hematopoietic
HWBCH13	SEQ ID NO:855		Immune/Hematopoietic
HWBCH13	SEQ ID NO:856		Immune/Hematopoietic
HWBCH13	SEQ ID NO:857		Immune/Hematopoietic
HWBCM79	SEQ ID NO:858		Immune/Hematopoietic
HWBCV72	SEQ ID NO:859		Cancer
HWBCV72	SEQ ID NO:860		Cancer
HWBCV72	SEQ ID NO:861		Cancer
HWBCV72	SEQ ID NO:862		Cancer
HWBDM62	SEQ ID NO:863		Endocrine, Immune/Hematopoietic

HWBDM62	SEQ ID NO:864		Endocrine, Immune/Hematopoietic
HWBDM62	SEQ ID NO:865		Endocrine, Immune/Hematopoietic
HWBDM62	SEQ ID NO:866		Endocrine, Immune/Hematopoietic
HMTAL77	SEQ ID NO:867		Cancer
HMTAL77	SEQ ID NO:868		Cancer
HDPRH52	SEQ ID NO:869		Cancer
HDPRH52	SEQ ID NO:870		Cancer
HDPSB18	SEQ ID NO:871		Cancer
HDPSB18	SEQ ID NO:872		Cancer
HDPSB18	SEQ ID NO:873		Cancer
HDPSB18	SEQ ID NO:874		Cancer
HDPSH53	SEQ ID NO:875		Immune/Hematopoietic, Reproductive
HDPSH53	SEQ ID NO:876		Immune/Hematopoietic, Reproductive
HDPLO25	SEQ ID NO:877		Cancer
HDPLO25	SEQ ID NO:878		Cancer
HDPLO25	SEQ ID NO:879		Cancer
HDPRN70	SEQ ID NO:880		Immune/Hematopoietic
HDPRN70	SEQ ID NO:881		Immune/Hematopoietic
HDPTW24	SEQ ID NO:882		Immune/Hematopoietic
HDPTW65	SEQ ID NO:883		Excretory
HDPTW65	SEQ ID NO:884		Excretory
HDPTW65	SEQ ID NO:885		Excretory
HDPWN93	SEQ ID NO:886		Cancer
HDPWN93	SEQ ID NO:887		Cancer
HDPWN93	SEQ ID NO:888		Cancer
HDPXY01	SEQ ID NO:889		Cancer
HDPXY01	SEQ ID NO:890		Cancer
HDPXY01	SEQ ID NO:891		Cancer
HDPXY01	SEQ ID NO:892		Cancer
HWHPM16	SEQ ID NO:893		Cancer
HWHPM16	SEQ ID NO:894		Cancer
HLDQA07	SEQ ID NO:895		Digestive
HLDQA07	SEQ ID NO:896		Digestive
HDTFE17	SEQ ID NO:897		Cancer
HDTFE17	SEQ ID NO:898		Cancer
HDTFE17	SEQ ID NO:899		Cancer
HWDAD17	SEQ ID NO:900		Cancer
HWDAD17	SEQ ID NO:901		Cancer
HWEAC77	SEQ ID NO:902		Connective/Epithelial
HWEAC77	SEQ ID NO:903		Connective/Epithelial
HWBEM18	SEQ ID NO:904		Cancer
HWBEM18	SEQ ID NO:905		Cancer
HWBEM18	SEQ ID NO:906		Cancer
HWBFE57	SEQ ID NO:907		Cancer
HWBFE57	SEQ ID NO:908		Cancer
HWBFE57	SEQ ID NO:909		Cancer
HOHDF66	SEQ ID NO:910		Musculoskeletal
HOHDF66	SEQ ID NO:911		Musculoskeletal
HOHDF66	SEQ ID NO:912		Musculoskeletal
HOHDC86	SEQ ID NO:913		Musculoskeletal



HOHDC86	SEQ ID NO:914		Musculoskeletal
HOHDC86	SEQ ID NO:915		Musculoskeletal
HRADO01	SEQ ID NO:916		Excretory
HRADO01	SEQ ID NO:917		Excretory
HRADO01	SEQ ID NO:918		Excretory
HRAEE45	SEQ ID NO:919		Connective/Epithelial, Excretory, Immune/Hematopoietic
HRAEE45	SEQ ID NO:920		Connective/Epithelial, Excretory, Immune/Hematopoietic
HRAEE45	SEQ ID NO:921		Connective/Epithelial, Excretory, Immune/Hematopoietic
HRAEH37	SEQ ID NO:922		Cancer
HRAEH37	SEQ ID NO:923		Cancer
HRAEH37	SEQ ID NO:924		Cancer
HWDH38	SEQ ID NO:925		Cancer
HWDH38	SEQ ID NO:926		Cancer
HLWCP78	SEQ ID NO:927		Cancer
HLWCP78	SEQ ID NO:928		Cancer
HLWCP78	SEQ ID NO:929		Cancer
HLWCP78	SEQ ID NO:930		Cancer
HTJML75	SEQ ID NO:931		Cancer
HTJML75	SEQ ID NO:932		Cancer
HTJNX29	SEQ ID NO:933		Connective/Epithelial, Digestive, Immune/Hematopoietic
HTJNX29	SEQ ID NO:934		Connective/Epithelial, Digestive, Immune/Hematopoietic
HTJNX29	SEQ ID NO:935		Connective/Epithelial, Digestive, Immune/Hematopoietic
HHESQ62	SEQ ID NO:936		Immune/Hematopoietic
HHESQ62	SEQ ID NO:937		Immune/Hematopoietic
HHESQ62	SEQ ID NO:938		Immune/Hematopoietic
HHESQ62	SEQ ID NO:939		Immune/Hematopoietic
HHESQ62	SEQ ID NO:940		Immune/Hematopoietic
HDQGO29	SEQ ID NO:941		Immune/Hematopoietic
HDQGO29	SEQ ID NO:942		Immune/Hematopoietic
HDQGO29	SEQ ID NO:943		Immune/Hematopoietic
HDQGO29	SEQ ID NO:944		Immune/Hematopoietic
HDQHY04	SEQ ID NO:945		Cancer
HDQHY04	SEQ ID NO:946		Cancer
HDQHY04	SEQ ID NO:947		Cancer
HBXAB02	SEQ ID NO:948		Cancer
HBXAB02	SEQ ID NO:949		Cancer
HBXAB02	SEQ ID NO:950		Cancer
HCWAU23	SEQ ID NO:951		Immune/Hematopoietic
HCWAU23	SEQ ID NO:952		Immune/Hematopoietic
HCWAU23	SEQ ID NO:953		Immune/Hematopoietic
HBXAM53	SEQ ID NO:954		Cancer
HBXAM53	SEQ ID NO:955		Cancer
HBXAM53	SEQ ID NO:956		Cancer
HCWBP34	SEQ ID NO:957		Immune/Hematopoietic

HCWBP34	SEQ ID NO:958		Immune/Hematopoietic
HCWBP34	SEQ ID NO:959		Immune/Hematopoietic
HBXCT44	SEQ ID NO:960		Cancer
HBXCT44	SEQ ID NO:961		Cancer
HBXCT44	SEQ ID NO:962		Cancer
HBXCT44	SEQ ID NO:963		Cancer
HCWDY64	SEQ ID NO:964		Excretory, Immune/Hematopoietic
HCWDY64	SEQ ID NO:965		Excretory, Immune/Hematopoietic
HCWDY64	SEQ ID NO:966		Excretory, Immune/Hematopoietic
HCWEB58	SEQ ID NO:967		Cancer
HCWEB58	SEQ ID NO:968		Cancer
HBXED80	SEQ ID NO:969		Immune/Hematopoietic, Neural/Sensory
HBXED80	SEQ ID NO:970		Immune/Hematopoietic, Neural/Sensory
HBXED80	SEQ ID NO:971		Immune/Hematopoietic, Neural/Sensory
HBXED80	SEQ ID NO:972		Immune/Hematopoietic, Neural/Sensory
HCWFT79	SEQ ID NO:973		Immune/Hematopoietic
HCWFT79	SEQ ID NO:974		Immune/Hematopoietic
HCWFT79	SEQ ID NO:975		Immune/Hematopoietic
HCWFT79	SEQ ID NO:976		Cancer
HCWFT79	SEQ ID NO:977		Cancer
HCWFT79	SEQ ID NO:978		Cancer
HBXFZ38	SEQ ID NO:979		Cancer
HBXFZ38	SEQ ID NO:980		Cancer
HBXFZ38	SEQ ID NO:981		Cancer
HCUGC55	SEQ ID NO:982		Immune/Hematopoietic
HCUGC55	SEQ ID NO:983		Immune/Hematopoietic
HCUGC55	SEQ ID NO:984		Immune/Hematopoietic
HCWGU37	SEQ ID NO:985		Immune/Hematopoietic, Neural/Sensory, Reproductive
HCWGU37	SEQ ID NO:986		Immune/Hematopoietic, Neural/Sensory, Reproductive
HCWHV88	SEQ ID NO:987		Digestive, Immune/Hematopoietic, Reproductive
HCWHV88	SEQ ID NO:988		Digestive, Immune/Hematopoietic, Reproductive
HCWHX82	SEQ ID NO:989		Immune/Hematopoietic, Neural/Sensory
HCWHX82	SEQ ID NO:990		Immune/Hematopoietic, Neural/Sensory
HCWHX82	SEQ ID NO:991		Immune/Hematopoietic, Neural/Sensory
HCWFZ59	SEQ ID NO:992		Immune/Hematopoietic
HCWFZ59	SEQ ID NO:993		Immune/Hematopoietic
HCWFZ59	SEQ ID NO:994		Immune/Hematopoietic
HCWFZ59	SEQ ID NO:995		Immune/Hematopoietic

HBWCB95	SEQ ID NO:996		Neural/Sensory
HBWCB95	SEQ ID NO:997		Neural/Sensory
HBWCB95	SEQ ID NO:998		Neural/Sensory
HBWBR94	SEQ ID NO:999		Neural/Sensory
HBWBR94	SEQ ID NO:1000		Neural/Sensory
HBWBR94	SEQ ID NO:1001		Neural/Sensory
HBWCF75	SEQ ID NO:1002		Neural/Sensory
HBWCF75	SEQ ID NO:1003		Neural/Sensory
HBWCF75	SEQ ID NO:1004		Neural/Sensory
HBWCM83	SEQ ID NO:1005		Digestive, Immune/Hematopoietic, Neural/Sensory
HBWCM83	SEQ ID NO:1006		Digestive, Immune/Hematopoietic, Neural/Sensory
HBWCM83	SEQ ID NO:1007		Digestive, Immune/Hematopoietic, Neural/Sensory
HBWCM83	SEQ ID NO:1008		Digestive, Immune/Hematopoietic, Neural/Sensory
HRSMQ86	SEQ ID NO:1009		Cancer
HRSMQ86	SEQ ID NO:1010		Cancer
HFCAA91	SEQ ID NO:1011		Neural/Sensory
HFCAA91	SEQ ID NO:1012		Neural/Sensory
HFCAA91	SEQ ID NO:1013		Neural/Sensory
HFCAL39	SEQ ID NO:1014		Cancer
HFCAL39	SEQ ID NO:1015		Cancer
HFCAL39	SEQ ID NO:1016		Cancer
HCEBN44	SEQ ID NO:1017		Neural/Sensory
HCEBN44	SEQ ID NO:1018		Neural/Sensory
HHFCP32	SEQ ID NO:1019		Cancer
HGBAJ60	SEQ ID NO:1020		Cancer
HGBAJ60	SEQ ID NO:1021		Cancer
HHFCW75	SEQ ID NO:1022		Cardiovascular
HHFCW75	SEQ ID NO:1023		Cardiovascular
HHFCW75	SEQ ID NO:1024		Cardiovascular
HHFCZ67	SEQ ID NO:1025		Cancer
HHFCZ67	SEQ ID NO:1026		Cancer
HHFCZ67	SEQ ID NO:1027		Cancer
HHFCZ67	SEQ ID NO:1028		Cancer
HJBAR01	SEQ ID NO:1029		Cancer
HJBAR01	SEQ ID NO:1030		Cancer
HETAR42	SEQ ID NO:1031		Cancer
HETAR42	SEQ ID NO:1032		Cancer
HETAR42	SEQ ID NO:1033		Cancer
HETAR42	SEQ ID NO:1034		Cancer
HETAM53	SEQ ID NO:1035		Cancer
HETAM53	SEQ ID NO:1036		Cancer
HETAM53	SEQ ID NO:1037		Cancer
HETAM53	SEQ ID NO:1038		Cancer
HETAM53	SEQ ID NO:1039		Cancer
HTPBG16	SEQ ID NO:1040		Digestive, Immune/Hematopoietic
HTPBG16	SEQ ID NO:1041		Digestive, Immune/Hematopoietic

HTPBG16	SEQ ID NO:1042		Digestive, Immune/Hematopoietic
HJAAJ58	SEQ ID NO:1043		Immune/Hematopoietic
HJAAJ58	SEQ ID NO:1044		Immune/Hematopoietic
HJAAJ58	SEQ ID NO:1045		Immune/Hematopoietic
HJAAJ58	SEQ ID NO:1046		Immune/Hematopoietic
HSBBT12	SEQ ID NO:1047		Cancer
HSBBT12	SEQ ID NO:1048		Cancer
HSBBT12	SEQ ID NO:1049		Cancer
HE8MH77	SEQ ID NO:1050		Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HE8MH77	SEQ ID NO:1051		Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HE8MH77	SEQ ID NO:1052		Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HTEDJ85	SEQ ID NO:1053		Cancer
HTEDJ85	SEQ ID NO:1054		Cancer
HTEDJ85	SEQ ID NO:1055		Cancer
HTEDJ85	SEQ ID NO:1056		Cancer
HOVAF78	SEQ ID NO:1057		Cancer
HOVAF78	SEQ ID NO:1058		Cancer
HOVAF78	SEQ ID NO:1059		Cancer
HOVAF78	SEQ ID NO:1060		Cancer
HOVAF78	SEQ ID NO:1061		Cancer
HHGDE24	SEQ ID NO:1062		Cancer
HHGDE24	SEQ ID NO:1063		Cancer
HHGDE24	SEQ ID NO:1064		Cancer
HOUFU35	SEQ ID NO:1065		Connective/Epithelial
HOUFU35	SEQ ID NO:1066		Connective/Epithelial
HOUFU35	SEQ ID NO:1067		Connective/Epithelial
HOUFU35	SEQ ID NO:1068		Connective/Epithelial
HSIGD79	SEQ ID NO:1069		Cancer
HSIGD79	SEQ ID NO:1070		Cancer
HCQCT05	SEQ ID NO:1071		Digestive, Endocrine, Reproductive
HCQCT05	SEQ ID NO:1072		Digestive, Endocrine, Reproductive
HMVDL30	SEQ ID NO:1073		Cancer
HMVDL30	SEQ ID NO:1074		Cancer
HMVDL30	SEQ ID NO:1075		Cancer
HMVDL30	SEQ ID NO:1076		Cancer
HTGGO35	SEQ ID NO:1077		Cancer
HTGGO35	SEQ ID NO:1078		Cancer
HTGGO35	SEQ ID NO:1079		Cancer
HCLBW50	SEQ ID NO:1080		Cancer
HCLBW50	SEQ ID NO:1081		Cancer
HCLBW50	SEQ ID NO:1082		Cancer
HCLBW50	SEQ ID NO:1083		Cancer
HWLEV32	SEQ ID NO:1084		Cancer
HWLEV32	SEQ ID NO:1085		Cancer
HWLEV32	SEQ ID NO:1086		Cancer



HNGNN78	SEQ ID NO:1137		Cancer
HTLHC59	SEQ ID NO:1138		Digestive, Reproductive
HTLHC59	SEQ ID NO:1139		Digestive, Reproductive
HTLJF15	SEQ ID NO:1140		Immune/Hematopoietic, Reproductive
HTLJF15	SEQ ID NO:1141		Immune/Hematopoietic, Reproductive
HTLJF15	SEQ ID NO:1142		Immune/Hematopoietic, Reproductive
HPJCC05	SEQ ID NO:1143		Reproductive
HPJCC05	SEQ ID NO:1144		Reproductive
HPJCC05	SEQ ID NO:1145		Reproductive
HDPVW11	SEQ ID NO:1146		Cancer
HDPVW11	SEQ ID NO:1147		Cancer
HDPWP69	SEQ ID NO:1148		Cancer
HDPWP69	SEQ ID NO:1149		Cancer
HDPWP69	SEQ ID NO:1150		Cancer
HWHHD11	SEQ ID NO:1151		Cancer
HWHHD11	SEQ ID NO:1152		Cancer
HWHHD11	SEQ ID NO:1153		Cancer
HBIMT93	SEQ ID NO:1154		Cancer
HBIMT93	SEQ ID NO:1155		Cancer
HBIMT93	SEQ ID NO:1156		Cancer
HHATA33	SEQ ID NO:1157		Cancer
HHATA33	SEQ ID NO:1158		Cancer
HNTDL21	SEQ ID NO:1159		Cancer
HNTDL21	SEQ ID NO:1160		Cancer
HNTNK95	SEQ ID NO:1161		Cancer
HNTNK95	SEQ ID NO:1162		Cancer
HNTNK95	SEQ ID NO:1163		Cancer
HWEAD64	SEQ ID NO:1164		Cancer
HWEAD64	SEQ ID NO:1165		Cancer
HWLHZ28	SEQ ID NO:1166		Cancer
HWLHZ28	SEQ ID NO:1167		Cancer
HWLHZ28	SEQ ID NO:1168		Cancer
HWLHZ28	SEQ ID NO:1169		Cancer
HWLJE21	SEQ ID NO:1170		Cancer
HWLJE21	SEQ ID NO:1171		Cancer
HWLJE21	SEQ ID NO:1172		Cancer
HPASD51	SEQ ID NO:1173		Digestive, Excretory, Reproductive
HPASD51	SEQ ID NO:1174		Digestive, Excretory, Reproductive
HSICQ15	SEQ ID NO:1175		Cancer
HSICQ15	SEQ ID NO:1176		Cancer
HFEBP27	SEQ ID NO:1177		Cancer
HFEBP27	SEQ ID NO:1178		Cancer
HFEBP27	SEQ ID NO:1179		Cancer
HTOIZ28	SEQ ID NO:1180		Cancer
HTOIZ28	SEQ ID NO:1181		Cancer
HTOIZ28	SEQ ID NO:1182		Cancer
HTOIZ28	SEQ ID NO:1183		Cancer

HCE4L28	SEQ ID NO:1184		Cancer
HCE4L28	SEQ ID NO:1185		Cancer
HCE4L28	SEQ ID NO:1186		Cancer
HCE4L28	SEQ ID NO:1187		Cancer
HFVGM16	SEQ ID NO:1188		Cancer
HFVGM16	SEQ ID NO:1189		Cancer
HPMGR66	SEQ ID NO:1190		Cancer
HPMGR66	SEQ ID NO:1191		Cancer
HLYDU43	SEQ ID NO:1192		Cancer
HLYDU43	SEQ ID NO:1193		Cancer
HPJCK10	SEQ ID NO:1194		Cancer
HPJCK10	SEQ ID NO:1195		Cancer
HT5EK75	SEQ ID NO:1196		Cancer
HT5EK75	SEQ ID NO:1197		Cancer
HT5EK75	SEQ ID NO:1198		Cancer
HWLEZ82	SEQ ID NO:1199		Cancer
HWLEZ82	SEQ ID NO:1200		Cancer
HWLEZ82	SEQ ID NO:1201		Cancer
HWLEZ82	SEQ ID NO:1202		Cancer
HDRMB11	SEQ ID NO:1203		Digestive
HDRMB11	SEQ ID NO:1204		Digestive
HDRMB11	SEQ ID NO:1205		Digestive
HCRNC80	SEQ ID NO:1206		Cancer
HCRNC80	SEQ ID NO:1207		Cancer
HCRNC80	SEQ ID NO:1208		Cancer
HCRNF14	SEQ ID NO:1209		Cancer
HCRNF14	SEQ ID NO:1210		Cancer
HCRNF14	SEQ ID NO:1211		Cancer
HE9PF45	SEQ ID NO:1212		Cancer
HE9PF45	SEQ ID NO:1213		Cancer
HE9PF45	SEQ ID NO:1214		Cancer
HISEN93	SEQ ID NO:1215		Cancer
HISEN93	SEQ ID NO:1216		Cancer
HISEN93	SEQ ID NO:1217		Cancer
HODEA51	SEQ ID NO:1218		Cancer
HODEA51	SEQ ID NO:1219		Cancer
HODEA51	SEQ ID NO:1220		Cancer
HODEA51	SEQ ID NO:1221		Cancer
HUSJN32	SEQ ID NO:1222		Cancer
HUSJN32	SEQ ID NO:1223		Cancer
HUSJN32	SEQ ID NO:1224		Cancer
HNGNW50	SEQ ID NO:1225		Immune/Hematopoietic, Mixed Fetal, Reproductive
HNGNW50	SEQ ID NO:1226		Immune/Hematopoietic, Mixed Fetal, Reproductive
HNGNW50	SEQ ID NO:1227		Immune/Hematopoietic, Mixed Fetal, Reproductive
HUVFB80	SEQ ID NO:1228		Cancer
HUVFB80	SEQ ID NO:1229		Cancer
HFIDQ92	SEQ ID NO:1230		Cancer
HFIDQ92	SEQ ID NO:1231		Cancer
HFIDQ92	SEQ ID NO:1232		Cancer
HTLJC07	SEQ ID NO:1233		Immune/Hematopoietic,

			Neural/Sensory, Reproductive
HTLJC07	SEQ ID NO:1234		Immune/Hematopoietic, Neural/Sensory, Reproductive
HTLJC07	SEQ ID NO:1235		Immune/Hematopoietic, Neural/Sensory, Reproductive
HMSOW51	SEQ ID NO:1236		Cancer
HMSOW51	SEQ ID NO:1237		Cancer
HPJEZ38	SEQ ID NO:1238		Cancer
HPJEZ38	SEQ ID NO:1239		Cancer
HPJEZ38	SEQ ID NO:1240		Cancer
HTAGN51	SEQ ID NO:1241		Immune/Hematopoietic, Neural/Sensory, Reproductive
HTAGN51	SEQ ID NO:1242		Immune/Hematopoietic, Neural/Sensory, Reproductive
HHFLH45	SEQ ID NO:1243		Cardiovascular, Reproductive
HHFLH45	SEQ ID NO:1244		Cardiovascular, Reproductive
HHFLH45	SEQ ID NO:1245		Cardiovascular, Reproductive
HFKLE15	SEQ ID NO:1246		Cancer
HFKLE15	SEQ ID NO:1247		Cancer
HNSAA27	SEQ ID NO:1248		Digestive
HNSAA27	SEQ ID NO:1249		Digestive
HUVFY29	SEQ ID NO:1250		Cancer
HUVFY29	SEQ ID NO:1251		Cancer
HAVUR23	SEQ ID NO:1252		Neural/Sensory
HAVUR23	SEQ ID NO:1253		Neural/Sensory
HTPIH83	SEQ ID NO:1254		Digestive, Reproductive
HTPIH83	SEQ ID NO:1255		Digestive, Reproductive
HTPIH83	SEQ ID NO:1256		Digestive, Reproductive
HUCNC61	SEQ ID NO:1257		Cancer
HIDAF73	SEQ ID NO:1258		Cancer
HIDAF73	SEQ ID NO:1259		Cancer
HIDAF73	SEQ ID NO:1260		Cancer
HOFMA42	SEQ ID NO:1261		Reproductive
HOFMA42	SEQ ID NO:1262		Reproductive
HKABW11	SEQ ID NO:1263		Cancer
HKABW11	SEQ ID NO:1264		Cancer
HWBAO29	SEQ ID NO:1265		Immune/Hematopoietic, Reproductive
HWBAO29	SEQ ID NO:1266		Immune/Hematopoietic, Reproductive
HWBAO29	SEQ ID NO:1267		Immune/Hematopoietic, Reproductive
HDPTM61	SEQ ID NO:1268		Digestive, Immune/Hematopoietic
HDPTM61	SEQ ID NO:1269		Digestive,



			Immune/Hematopoietic
HKAHL26	SEQ ID NO:1270		Cancer
HKAHL26	SEQ ID NO:1271		Cancer
HDQHC29	SEQ ID NO:1272		Cancer
HDQHQ91	SEQ ID NO:1273		Cancer
HDQHQ91	SEQ ID NO:1274		Cancer
HDQHQ91	SEQ ID NO:1275		Cancer
HDTLR06	SEQ ID NO:1276		Cancer
HDTLR06	SEQ ID NO:1277		Cancer
HNTDE84	SEQ ID NO:1278		Cancer
HNTDE84	SEQ ID NO:1279		Cancer
HWAFT87	SEQ ID NO:1280		Cardiovascular, Immune/Hematopoietic
HWAFT87	SEQ ID NO:1281		Cardiovascular, Immune/Hematopoietic
HWAFT87	SEQ ID NO:1282		Cardiovascular, Immune/Hematopoietic
HOGCE48	SEQ ID NO:1283		Cancer
HOGCE48	SEQ ID NO:1284		Cancer
HBINS58	SEQ ID NO:1285		Connective/Epithelial, Reproductive
HBINS58	SEQ ID NO:1286		Connective/Epithelial, Reproductive
HHAUQ28	SEQ ID NO:1287		Cancer
HHAUQ28	SEQ ID NO:1288		Cancer
HBIOH81	SEQ ID NO:1289		Cancer
HBIOH81	SEQ ID NO:1290		Cancer
HOGDP46	SEQ ID NO:1291		Cancer
HOGDP46	SEQ ID NO:1292		Cancer
HWHIH10	SEQ ID NO:1293		Cancer
HWHIH10	SEQ ID NO:1294		Cancer
HCWCT62	SEQ ID NO:1295		Immune/Hematopoietic
HCWCT62	SEQ ID NO:1296		Immune/Hematopoietic
HCWCT62	SEQ ID NO:1297		Immune/Hematopoietic
HBXCL50	SEQ ID NO:1298		Digestive, Excretory, Neural/Sensory
HBXCL50	SEQ ID NO:1299		Digestive, Excretory, Neural/Sensory
HACAA29	SEQ ID NO:1300		Cancer
HACAA29	SEQ ID NO:1301		Cancer
HAJAR23	SEQ ID NO:1302		Cancer
HAJAR23	SEQ ID NO:1303		Cancer
HAJAR23	SEQ ID NO:1304		Cancer
HDPQN12	SEQ ID NO:1305		Cancer
HDPQN12	SEQ ID NO:1306		Cancer
HDQFN31	SEQ ID NO:1307		Cancer
HDQFN31	SEQ ID NO:1308		Cancer
HDQIH54	SEQ ID NO:1309		Immune/Hematopoietic
HDQIH54	SEQ ID NO:1310		Immune/Hematopoietic
HETKL27	SEQ ID NO:1311		Cancer
HETKL27	SEQ ID NO:1312		Cancer
HETKL27	SEQ ID NO:1313		Cancer
HETKL27	SEQ ID NO:1314		Cancer
HFIHQ89	SEQ ID NO:1315		Cancer

HFIHQ89	SEQ ID NO:1316	Cancer
HFKHW50	SEQ ID NO:1317	Cancer
HFKHW50	SEQ ID NO:1318	Cancer
HFKHW50	SEQ ID NO:1319	Cancer
HMEJL08	SEQ ID NO:1320	Cancer
HMEJL08	SEQ ID NO:1321	Cancer
HMEJL08	SEQ ID NO:1322	Cancer
HMSCT72	SEQ ID NO:1323	Connective/Epithelial, Immune/Hematopoietic
HMSCT72	SEQ ID NO:1324	Connective/Epithelial, Immune/Hematopoietic
HMSCT72	SEQ ID NO:1325	Connective/Epithelial, Immune/Hematopoietic
HPJEX20	SEQ ID NO:1326	Immune/Hematopoietic, Reproductive
HPJEX20	SEQ ID NO:1327	Immune/Hematopoietic, Reproductive
HPJEX20	SEQ ID NO:1328	Immune/Hematopoietic, Reproductive
HPJEX20	SEQ ID NO:1329	Immune/Hematopoietic, Reproductive
HSLGM21	SEQ ID NO:1330	Cancer
HSLGM21	SEQ ID NO:1331	Cancer
HSLHI86	SEQ ID NO:1332	Cancer
HSLHI86	SEQ ID NO:1333	Cancer
HSLHI86	SEQ ID NO:1334	Cancer
HSLHI86	SEQ ID NO:1335	Cancer
HUCNP80	SEQ ID NO:1336	Cancer
HUCNP80	SEQ ID NO:1337	Cancer
HBINK72	SEQ ID NO:1338	Cancer
HBINK72	SEQ ID NO:1339	Cancer
HBINK72	SEQ ID NO:1340	Cancer
HIABC55	SEQ ID NO:1341	Cancer
HIABC55	SEQ ID NO:1342	Cancer
HIABC55	SEQ ID NO:1343	Cancer
HIABC55	SEQ ID NO:1344	Cancer
HGBAR55	SEQ ID NO:1345	Cancer
HGBAR55	SEQ ID NO:1346	Cancer
HGBAR55	SEQ ID NO:1347	Cancer
HE2FE45	SEQ ID NO:1348	Cancer
HE2FE45	SEQ ID NO:1349	Cancer
HE2FE45	SEQ ID NO:1350	Cancer
HMRAD54	SEQ ID NO:1351	Cancer
HMRAD54	SEQ ID NO:1352	Cancer
HMRAD54	SEQ ID NO:1353	Cancer
HCEFB80	SEQ ID NO:1354	Cancer
HCEFB80	SEQ ID NO:1355	Cancer
HFTBN23	SEQ ID NO:1356	Cancer
HFTBN23	SEQ ID NO:1357	Cancer
HFTBN23	SEQ ID NO:1358	Cancer
HFTBQ52	SEQ ID NO:1359	Cancer
HFTBQ52	SEQ ID NO:1360	Cancer
HMEEJ79	SEQ ID NO:1361	Cardiovascular, Neural/Sensory, Reproductive
HMEEJ79	SEQ ID NO:1362	Cardiovascular,

			Neural/Sensory, Reproductive
HROAJ39	SEQ ID NO:1363		Cancer
HROAJ39	SEQ ID NO:1364		Cancer
HROAJ39	SEQ ID NO:1365		Cancer
HFEBV76	SEQ ID NO:1366		Cancer
HFEBV76	SEQ ID NO:1367		Cancer
HTADC09	SEQ ID NO:1368		Cancer
HTADC09	SEQ ID NO:1369		Cancer
HFXBJ12	SEQ ID NO:1370		Neural/Sensory
HFXBJ12	SEQ ID NO:1371		Neural/Sensory
HFXBJ12	SEQ ID NO:1372		Neural/Sensory
HMHBN86	SEQ ID NO:1373		Cancer
HMHBN86	SEQ ID NO:1374		Cancer
HMHBN86	SEQ ID NO:1375		Cancer
HFKFL92	SEQ ID NO:1376		Cancer
HFKFL92	SEQ ID NO:1377		Cancer
HFKFL92	SEQ ID NO:1378		Cancer
HASAW52	SEQ ID NO:1379		Cancer
HTLDT76	SEQ ID NO:1380		Cardiovascular, Neural/Sensory, Reproductive
HTLDT76	SEQ ID NO:1381		Cardiovascular, Neural/Sensory, Reproductive
HTLDT76	SEQ ID NO:1382		Cardiovascular, Neural/Sensory, Reproductive
HTLEC34	SEQ ID NO:1383		Immune/Hematopoietic, Neural/Sensory, Reproductive
HTLEC34	SEQ ID NO:1384		Immune/Hematopoietic, Neural/Sensory, Reproductive
HNHFB60	SEQ ID NO:1385		Immune/Hematopoietic
HNHFB60	SEQ ID NO:1386		Immune/Hematopoietic
HNHFB60	SEQ ID NO:1387		Immune/Hematopoietic
H2CBK33	SEQ ID NO:1388		Cancer
H2CBK33	SEQ ID NO:1389		Cancer
H2CBK33	SEQ ID NO:1390		Cancer
HNGEY29	SEQ ID NO:1391		Cancer
HNGEY29	SEQ ID NO:1392		Cancer
HUSFE58	SEQ ID NO:1393		Cancer
HUSFE58	SEQ ID NO:1394		Cancer
HMSHS36	SEQ ID NO:1395		Immune/Hematopoietic
HMSHS36	SEQ ID NO:1396		Immune/Hematopoietic
HMSKC10	SEQ ID NO:1397		Immune/Hematopoietic
HMSKC10	SEQ ID NO:1398		Immune/Hematopoietic
HMSKC10	SEQ ID NO:1399		Immune/Hematopoietic
HSLGU75	SEQ ID NO:1400		Cancer
HSLGU75	SEQ ID NO:1401		Cancer
HSLGU75	SEQ ID NO:1402		Cancer
HDABU01	SEQ ID NO:1403		Cancer
HDABU01	SEQ ID NO:1404		Cancer
HDABU01	SEQ ID NO:1405		Cancer
HADGD17	SEQ ID NO:1406		Connective/Epithelial

HADGD17	SEQ ID NO:1407		Connective/Epithelial
HADGD17	SEQ ID NO:1408		Connective/Epithelial
HFIUE67	SEQ ID NO:1409		Cancer
HKGAM29	SEQ ID NO:1410		Cancer
HACBD86	SEQ ID NO:1411		Cancer
HACBD86	SEQ ID NO:1412		Cancer
HACBD86	SEQ ID NO:1413		Cancer
HEGAK23	SEQ ID NO:1414		Cancer
HEGAK23	SEQ ID NO:1415		Cancer
HEGAK23	SEQ ID NO:1416		Cancer
HEGAK23	SEQ ID NO:1417		Cancer
HCHAR90	SEQ ID NO:1418		Cancer
HCHAR90	SEQ ID NO:1419		Cancer
HCHAR90	SEQ ID NO:1420		Cancer
HLYCK27	SEQ ID NO:1421		Immune/Hematopoietic
HMVBP38	SEQ ID NO:1422		Cancer
HMVBP38	SEQ ID NO:1423		Cancer
HMVBP38	SEQ ID NO:1424		Cancer
HFACI31	SEQ ID NO:1425		Neural/Sensory
HFACI31	SEQ ID NO:1426		Neural/Sensory
HFACI31	SEQ ID NO:1427		Neural/Sensory
HBJKC04	SEQ ID NO:1428		Immune/Hematopoietic
HBJKC04	SEQ ID NO:1429		Immune/Hematopoietic
HBJKC04	SEQ ID NO:1430		Immune/Hematopoietic
HBJIT60	SEQ ID NO:1431		Immune/Hematopoietic
HBJIT60	SEQ ID NO:1432		Immune/Hematopoietic
HBJIT60	SEQ ID NO:1433		Immune/Hematopoietic
HPJBK03	SEQ ID NO:1434		Cancer
HPJBK03	SEQ ID NO:1435		Cancer
HPJCL22	SEQ ID NO:1436		Cancer
HPJCL22	SEQ ID NO:1437		Cancer
HPJCL22	SEQ ID NO:1438		Cancer
HTWJB71	SEQ ID NO:1439		Immune/Hematopoietic, Neural/Sensory
HNTOE45	SEQ ID NO:1440		Cancer
HNTOE45	SEQ ID NO:1441		Cancer
HNTRW30	SEQ ID NO:1442		Digestive, Immune/Hematopoietic, Mixed Fetal
HNTRW30	SEQ ID NO:1443		Digestive, Immune/Hematopoietic, Mixed Fetal
HCHPU32	SEQ ID NO:1444		Cancer
HCHPU32	SEQ ID NO:1445		Cancer
HCHPU32	SEQ ID NO:1446		Cancer
HGCNC48	SEQ ID NO:1447		Reproductive
HGCNC48	SEQ ID NO:1448		Reproductive
HLTHO84	SEQ ID NO:1449		Cancer
HSLIA81	SEQ ID NO:1450		Cancer
HSLIA81	SEQ ID NO:1451		Cancer
HSLIA81	SEQ ID NO:1452		Cancer
HSLIA81	SEQ ID NO:1453		Cancer
HBFMA07	SEQ ID NO:1454		Cancer
HBODE48	SEQ ID NO:1455		Digestive, Excretory, Immune/Hematopoietic

HBODE48	SEQ ID NO:1456		Digestive, Excretory, Immune/Hematopoietic
HBODE48	SEQ ID NO:1457		Digestive, Excretory, Immune/Hematopoietic
HBODE48	SEQ ID NO:1458		Digestive, Excretory, Immune/Hematopoietic
HCRME12	SEQ ID NO:1459		Cancer
HCRME12	SEQ ID NO:1460		Cancer
HBODQ16	SEQ ID NO:1461		Cancer
HBODQ16	SEQ ID NO:1462		Cancer
HASMB80	SEQ ID NO:1463		Cancer
HASMB80	SEQ ID NO:1464		Cancer
HBOEG11	SEQ ID NO:1465		Cancer
HBOEG11	SEQ ID NO:1466		Cancer
HCRNU76	SEQ ID NO:1467		Cancer
HCRNU76	SEQ ID NO:1468		Cancer
HAPSQ21	SEQ ID NO:1469		Reproductive, Respiratory
HAPSQ21	SEQ ID NO:1470		Reproductive, Respiratory
HAPSQ21	SEQ ID NO:1471		Reproductive, Respiratory
HWLNF33	SEQ ID NO:1472		Cancer
HWLNF33	SEQ ID NO:1473		Cancer
HE8QO53	SEQ ID NO:1474		Cancer
HE8QO53	SEQ ID NO:1475		Cancer
HE8QV67	SEQ ID NO:1476		Cancer
HE8QV67	SEQ ID NO:1477		Cancer
HE8TB68	SEQ ID NO:1478		Cancer
HE8TY90	SEQ ID NO:1479		Cancer
HE8TY90	SEQ ID NO:1480		Cancer
HE8TY90	SEQ ID NO:1481		Cancer
HE8TY90	SEQ ID NO:1482		Cancer
HETLM70	SEQ ID NO:1483		Digestive, Excretory, Reproductive
HETLM70	SEQ ID NO:1484		Digestive, Excretory, Reproductive
HETLM70	SEQ ID NO:1485		Digestive, Excretory, Reproductive
HISES66	SEQ ID NO:1486		Digestive, Reproductive
HISES66	SEQ ID NO:1487		Digestive, Reproductive
HISES66	SEQ ID NO:1488		Digestive, Reproductive
HTXKV29	SEQ ID NO:1489		Cancer
HTXKV29	SEQ ID NO:1490		Cancer
HTXKV29	SEQ ID NO:1491		Cancer
HTXLH48	SEQ ID NO:1492		Immune/Hematopoietic
HTXLH48	SEQ ID NO:1493		Immune/Hematopoietic



HODGL52	SEQ ID NO:1533		Cancer
HODGL52	SEQ ID NO:1534		Cancer
HTXNV67	SEQ ID NO:1535		Cancer
HTXNV67	SEQ ID NO:1536		Cancer
HTXNV67	SEQ ID NO:1537		Cancer
HOCNE30	SEQ ID NO:1538		Digestive, Musculoskeletal, Neural/Sensory
HOCNE30	SEQ ID NO:1539		Digestive, Musculoskeletal, Neural/Sensory
HOCNE30	SEQ ID NO:1540		Digestive, Musculoskeletal, Neural/Sensory
HMSOC30	SEQ ID NO:1541		Cancer
HMSOC30	SEQ ID NO:1542		Cancer
HWMAF61	SEQ ID NO:1543		Digestive
HWMAF61	SEQ ID NO:1544		Digestive
HWMAF61	SEQ ID NO:1545		Digestive
HWMAF61	SEQ ID NO:1546		Digestive
HWMAF61	SEQ ID NO:1547		Digestive
HWMAH36	SEQ ID NO:1548		Immune/Hematopoietic
HWMAH36	SEQ ID NO:1549		Immune/Hematopoietic
HXOAC69	SEQ ID NO:1550		Cancer
HXOAC69	SEQ ID NO:1551		Cancer
HPJDA23	SEQ ID NO:1552		Mixed Fetal, Neural/Sensory, Reproductive
HPJDA23	SEQ ID NO:1553		Mixed Fetal, Neural/Sensory, Reproductive
HPJEE14	SEQ ID NO:1554		Reproductive
HPJEE14	SEQ ID NO:1555		Reproductive
HPJEG57	SEQ ID NO:1556		Reproductive
HPJEG57	SEQ ID NO:1557		Reproductive
HPJEG57	SEQ ID NO:1558		Reproductive
HPJEV11	SEQ ID NO:1559		Cancer
HTTKT43	SEQ ID NO:1560		Cancer
HTTKT43	SEQ ID NO:1561		Cancer
HTTKT43	SEQ ID NO:1562		Cancer
HHFKM76	SEQ ID NO:1563		Cancer
HHFKM76	SEQ ID NO:1564		Cancer
HHFKM76	SEQ ID NO:1565		Cancer
HHFML08	SEQ ID NO:1566		Cardiovascular, Immune/Hematopoietic, Mixed Fetal
HHFML08	SEQ ID NO:1567		Cardiovascular, Immune/Hematopoietic, Mixed Fetal
HHFML08	SEQ ID NO:1568		Cardiovascular, Immune/Hematopoietic, Mixed Fetal
HTPFX69	SEQ ID NO:1569		Cancer
HTPFX69	SEQ ID NO:1570		Cancer
HTPFX69	SEQ ID NO:1571		Cancer
HTPFX69	SEQ ID NO:1572		Cancer

HFKLX38	SEQ ID NO:1573		Excretory, Respiratory
HFKLX38	SEQ ID NO:1574		Excretory, Respiratory
HFKLX38	SEQ ID NO:1575		Excretory, Respiratory
HFKME15	SEQ ID NO:1576		Excretory
HFKME15	SEQ ID NO:1577		Excretory
HUVFH14	SEQ ID NO:1578		Cancer
HUVFH14	SEQ ID NO:1579		Cancer
HUVFH14	SEQ ID NO:1580		Cancer
HE2KK74	SEQ ID NO:1581		Cancer
HE2KK74	SEQ ID NO:1582		Cancer
HE2KK74	SEQ ID NO:1583		Cancer
HMALI42	SEQ ID NO:1584		Immune/Hematopoietic
HE2LW65	SEQ ID NO:1585		Cancer
HE2LW65	SEQ ID NO:1586		Cancer
HE2LW65	SEQ ID NO:1587		Cancer
HTFOS57	SEQ ID NO:1588		Cancer
HTFOS57	SEQ ID NO:1589		Cancer
HTFOS57	SEQ ID NO:1590		Cancer
HUVHI35	SEQ ID NO:1591		Cancer
HUVHI35	SEQ ID NO:1592		Cancer
HUVHI35	SEQ ID NO:1593		Cancer
HUVHI35	SEQ ID NO:1594		Cancer
HTPHS66	SEQ ID NO:1595		Cancer
HTPHS66	SEQ ID NO:1596		Cancer
HTPHS66	SEQ ID NO:1597		Cancer
HHFOJ29	SEQ ID NO:1598		Cancer
HHFOJ29	SEQ ID NO:1599		Cancer
HHFOJ29	SEQ ID NO:1600		Cancer
HMAMI15	SEQ ID NO:1601		Cancer
HTXQM57	SEQ ID NO:1602		Immune/Hematopoietic, Mixed Fetal
HE2RO22	SEQ ID NO:1603		Mixed Fetal
HE2RO22	SEQ ID NO:1604		Mixed Fetal
HE2SI26	SEQ ID NO:1605		Cancer
HTXRE15	SEQ ID NO:1606		Cancer
HTXRE15	SEQ ID NO:1607		Cancer
HUCPD31	SEQ ID NO:1608		Cancer
HUCPD31	SEQ ID NO:1609		Cancer
HFPFA80	SEQ ID NO:1610		Neural/Sensory
HFPFA80	SEQ ID NO:1611		Neural/Sensory
HFPFA80	SEQ ID NO:1612		Neural/Sensory
HFPFA80	SEQ ID NO:1613		Neural/Sensory
HFPHB92	SEQ ID NO:1614		Excretory, Neural/Sensory
HFPHS77	SEQ ID NO:1615		Cancer
HFPHS77	SEQ ID NO:1616		Cancer
HFPHS77	SEQ ID NO:1617		Cancer
HIPAJ43	SEQ ID NO:1618		Cancer
HIPAJ43	SEQ ID NO:1619		Cancer
HDDMW90	SEQ ID NO:1620		Cancer
HDDMW90	SEQ ID NO:1621		Cancer
HBCPB32	SEQ ID NO:1622		Neural/Sensory, Reproductive



HFVKC95	SEQ ID NO:1623	Cancer
HFVKC95	SEQ ID NO:1624	Cancer
HFVKC95	SEQ ID NO:1625	Cancer
HCOMM91	SEQ ID NO:1626	Cancer
HCOMM91	SEQ ID NO:1627	Cancer
HVVAM64	SEQ ID NO:1628	Cancer
HVVAM64	SEQ ID NO:1629	Cancer
HVVAM64	SEQ ID NO:1630	Cancer
HNBUC50	SEQ ID NO:1631	Cancer
HNBUC50	SEQ ID NO:1632	Cancer
HNBUC50	SEQ ID NO:1633	Cancer
HNBUC50	SEQ ID NO:1634	Cancer
HUUDF48	SEQ ID NO:1635	Immune/Hematopoietic
HUUDF48	SEQ ID NO:1636	Immune/Hematopoietic
HBCQL32	SEQ ID NO:1637	Cancer
HBCQL32	SEQ ID NO:1638	Cancer
HCBND16	SEQ ID NO:1639	Cancer
HCBND16	SEQ ID NO:1640	Cancer
HNNBM45	SEQ ID NO:1641	Immune/Hematopoietic, Reproductive
HNNBM45	SEQ ID NO:1642	Immune/Hematopoietic, Reproductive
HWMGN33	SEQ ID NO:1643	Digestive
HWMGN33	SEQ ID NO:1644	Digestive
HWMLN52	SEQ ID NO:1645	Digestive, Immune/Hematopoietic
HWMLN52	SEQ ID NO:1646	Digestive, Immune/Hematopoietic
HWMLN52	SEQ ID NO:1647	Digestive, Immune/Hematopoietic
HVARW53	SEQ ID NO:1648	Digestive
HVARW53	SEQ ID NO:1649	Digestive
HAHFU44	SEQ ID NO:1650	Cardiovascular, Digestive, Musculoskeletal
HAHFU44	SEQ ID NO:1651	Cardiovascular, Digestive, Musculoskeletal
HAHFU44	SEQ ID NO:1652	Cardiovascular, Digestive, Musculoskeletal
HCOOS80	SEQ ID NO:1653	Cancer
HCOOS80	SEQ ID NO:1654	Cancer
HCOOS80	SEQ ID NO:1655	Cancer
HNKCO80	SEQ ID NO:1656	Cancer
HNKCO80	SEQ ID NO:1657	Cancer
HLTIP27	SEQ ID NO:1658	Immune/Hematopoietic
HLTIP27	SEQ ID NO:1659	Immune/Hematopoietic
HLTIP94	SEQ ID NO:1660	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HLTIP94	SEQ ID NO:1661	Immune/Hematopoietic, Mixed Fetal, Neural/Sensory
HLTIP94	SEQ ID NO:1662	Immune/Hematopoietic, Mixed Fetal,

			Neural/Sensory
HOCPM23	SEQ ID NO:1663		Reproductive
HOCPM23	SEQ ID NO:1664		Reproductive
HPDWP28	SEQ ID NO:1665		Reproductive
HPDWP28	SEQ ID NO:1666		Reproductive
HLCND09	SEQ ID NO:1667		Cancer
HLCND09	SEQ ID NO:1668		Cancer
HEEBI05	SEQ ID NO:1669		Digestive, Reproductive
HEEBB55	SEQ ID NO:1670		Cancer
HEEBB55	SEQ ID NO:1671		Cancer
HEEBB55	SEQ ID NO:1672		Cancer
HEGCL11	SEQ ID NO:1673		Cancer
HEGCL11	SEQ ID NO:1674		Cancer
HNTPB82	SEQ ID NO:1675		Cancer
HNTPB82	SEQ ID NO:1676		Cancer
HOFMM69	SEQ ID NO:1677		Reproductive
HOFMM69	SEQ ID NO:1678		Reproductive
HLDAB75	SEQ ID NO:1679		Cancer
HLDAB75	SEQ ID NO:1680		Cancer
HKACC80	SEQ ID NO:1681		Cancer
HKACC80	SEQ ID NO:1682		Cancer
HKACC80	SEQ ID NO:1683		Cancer
HKAEL28	SEQ ID NO:1684		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HKAEL28	SEQ ID NO:1685		Connective/Epithelial, Immune/Hematopoietic, Reproductive
HDPGT25	SEQ ID NO:1686		Cancer
HDPGT25	SEQ ID NO:1687		Cancer
HLWBT09	SEQ ID NO:1688		Excretory, Reproductive
HLWBT09	SEQ ID NO:1689		Excretory, Reproductive
HHEDN80	SEQ ID NO:1690		Cancer
HHEDN80	SEQ ID NO:1691		Cancer
HHEDN80	SEQ ID NO:1692		Cancer
HDFQB14	SEQ ID NO:1693		Immune/Hematopoietic, Neural/Sensory, Reproductive
HWAAW33	SEQ ID NO:1694		Cardiovascular, Immune/Hematopoietic, Musculoskeletal
HWAAW33	SEQ ID NO:1695		Cardiovascular, Immune/Hematopoietic, Musculoskeletal
HWABF47	SEQ ID NO:1696		Cancer
HWABF47	SEQ ID NO:1697		Cancer
HWABI12	SEQ ID NO:1698		Immune/Hematopoietic
HWABI12	SEQ ID NO:1699		Immune/Hematopoietic
HWBBT49	SEQ ID NO:1700		Cancer
HWBBT49	SEQ ID NO:1701		Cancer
HWBBT49	SEQ ID NO:1702		Cancer
HAMGG89	SEQ ID NO:1703		Immune/Hematopoietic, Neural/Sensory,

			Reproductive
HAMGG89	SEQ ID NO:1704		Immune/Hematopoietic, Neural/Sensory, Reproductive
HAJBW16	SEQ ID NO:1705		Neural/Sensory
HAJBW16	SEQ ID NO:1706		Neural/Sensory
HNTAI35	SEQ ID NO:1707		Cancer
HNTAI35	SEQ ID NO:1708		Cancer
HNTAI35	SEQ ID NO:1709		Cancer
HNTAI35	SEQ ID NO:1710		Cancer
HNTAI35	SEQ ID NO:1711		Cancer
HNTBN41	SEQ ID NO:1712		Immune/Hematopoietic
HNTBN41	SEQ ID NO:1713		Immune/Hematopoietic
HNTBN41	SEQ ID NO:1714		Immune/Hematopoietic
HNTBN41	SEQ ID NO:1715		Immune/Hematopoietic
HDPRJ60	SEQ ID NO:1716		Cancer
HDPRJ60	SEQ ID NO:1717		Cancer
HDPRJ60	SEQ ID NO:1718		Cancer
HDPSB01	SEQ ID NO:1719		Cancer
HDPSB01	SEQ ID NO:1720		Cancer
HDPSB01	SEQ ID NO:1721		Cancer
HDPSB01	SEQ ID NO:1722		Cancer
HDPSB01	SEQ ID NO:1723		Cancer
HDPTC31	SEQ ID NO:1724		Immune/Hematopoietic
HDPTC31	SEQ ID NO:1725		Immune/Hematopoietic
HDPTC31	SEQ ID NO:1726		Immune/Hematopoietic
HDPXL05	SEQ ID NO:1727		Immune/Hematopoietic, Reproductive
HDPXL05	SEQ ID NO:1728		Immune/Hematopoietic, Reproductive
HDPXL05	SEQ ID NO:1729		Immune/Hematopoietic, Reproductive
HDPXY88	SEQ ID NO:1730		Cancer
HDPXY88	SEQ ID NO:1731		Cancer
HDPXY88	SEQ ID NO:1732		Cancer
HLDQZ72	SEQ ID NO:1733		Cancer
HLDQZ72	SEQ ID NO:1734		Cancer
HLDQZ72	SEQ ID NO:1735		Cancer
HWBEV57	SEQ ID NO:1736		Immune/Hematopoietic
HWBEV57	SEQ ID NO:1737		Immune/Hematopoietic
HWBEV57	SEQ ID NO:1738		Immune/Hematopoietic
HAMHH20	SEQ ID NO:1739		Cancer
HAMHH20	SEQ ID NO:1740		Cancer
HDLAY18	SEQ ID NO:1741		Cancer
HDLAY18	SEQ ID NO:1742		Cancer
HKAHN23	SEQ ID NO:1743		Connective/Epithelial, Digestive, Mixed Fetal
HKAHN23	SEQ ID NO:1744		Connective/Epithelial, Digestive, Mixed Fetal
HKAJW28	SEQ ID NO:1745		Cancer
HKAJW28	SEQ ID NO:1746		Cancer
HDQFU73	SEQ ID NO:1747		Digestive, Immune/Hematopoietic
HDQFU73	SEQ ID NO:1748		Digestive,

			Immune/Hematopoietic
HDQFU73	SEQ ID NO:1749		Digestive, Immune/Hematopoietic
HDTKS69	SEQ ID NO:1750		Cancer
HSYDT06	SEQ ID NO:1751		Cancer
HSYDT06	SEQ ID NO:1752		Cancer
HSYDT06	SEQ ID NO:1753		Cancer
HSYDT06	SEQ ID NO:1754		Cancer
HNTEF28	SEQ ID NO:1755		Cancer
HNTEF28	SEQ ID NO:1756		Cancer
HNTEF53	SEQ ID NO:1757		Cancer
HNTEF53	SEQ ID NO:1758		Cancer
HNTEF53	SEQ ID NO:1759		Cancer
HNTEF53	SEQ ID NO:1760		Cancer
HDQFN60	SEQ ID NO:1761		Cancer
HDQFN60	SEQ ID NO:1762		Cancer
HHEXM06	SEQ ID NO:1763		Immune/Hematopoietic
HHEXM06	SEQ ID NO:1764		Immune/Hematopoietic
HBINU36	SEQ ID NO:1765		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HBINU36	SEQ ID NO:1766		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HBINU36	SEQ ID NO:1767		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HUJCQ39	SEQ ID NO:1768		Cancer
HUJCQ39	SEQ ID NO:1769		Cancer
HUJCQ39	SEQ ID NO:1770		Cancer
HCCCCG83	SEQ ID NO:1771		Cancer
HCCCCG83	SEQ ID NO:1772		Cancer
HCCCCG83	SEQ ID NO:1773		Cancer
HWHIM26	SEQ ID NO:1774		Connective/Epithelial, Immune/Hematopoietic
HWHIM26	SEQ ID NO:1775		Connective/Epithelial, Immune/Hematopoietic
HWHKC09	SEQ ID NO:1776		Cancer
HWHKC09	SEQ ID NO:1777		Cancer
HWHKC09	SEQ ID NO:1778		Cancer
HWHKC09	SEQ ID NO:1779		Cancer
HWHKR51	SEQ ID NO:1780		Cancer
HWHKR51	SEQ ID NO:1781		Cancer
HWHKR51	SEQ ID NO:1782		Cancer
HWHRL06	SEQ ID NO:1783		Cancer
HWHRL06	SEQ ID NO:1784		Cancer
HAZAD32	SEQ ID NO:1785		Cancer
HAZAD32	SEQ ID NO:1786		Cancer
HPAMY60	SEQ ID NO:1787		Excretory
HPAMY60	SEQ ID NO:1788		Excretory
HAOTS04	SEQ ID NO:1789		Reproductive
HAOTS04	SEQ ID NO:1790		Reproductive
HAZAP37	SEQ ID NO:1791		Reproductive
HKZAS29	SEQ ID NO:1792		Cancer
HKZAS29	SEQ ID NO:1793		Cancer
HOVJP29	SEQ ID NO:1794		Reproductive

HOVJP29	SEQ ID NO:1795		Reproductive
HWHSB53	SEQ ID NO:1796		Cancer
HWHSB53	SEQ ID NO:1797		Cancer
HKZBS01	SEQ ID NO:1798		Cancer
HKZBS01	SEQ ID NO:1799		Cancer
HWHSO13	SEQ ID NO:1800		Connective/Epithelial
HWHSO13	SEQ ID NO:1801		Connective/Epithelial
HKZCK47	SEQ ID NO:1802		Immune/Hematopoietic, Reproductive
HCUHQ40	SEQ ID NO:1803		Cancer
HCUHQ40	SEQ ID NO:1804		Cancer
HCUHQ40	SEQ ID NO:1805		Cancer
HPJCP79	SEQ ID NO:1806		Cancer
HPJCP79	SEQ ID NO:1807		Cancer
HPJCP79	SEQ ID NO:1808		Cancer
HPJCP79	SEQ ID NO:1809		Cancer
HFXDI56	SEQ ID NO:1810		Immune/Hematopoietic, Musculoskeletal, Neural/Sensory
HFXDI56	SEQ ID NO:1811		Immune/Hematopoietic, Musculoskeletal, Neural/Sensory
HFXDI56	SEQ ID NO:1812		Immune/Hematopoietic, Musculoskeletal, Neural/Sensory
HFXDI56	SEQ ID NO:1813		Immune/Hematopoietic, Musculoskeletal, Neural/Sensory
HRDEP41	SEQ ID NO:1814		Cancer
HRDEP41	SEQ ID NO:1815		Cancer
HTEGF16	SEQ ID NO:1816		Cancer
HTEGF16	SEQ ID NO:1817		Cancer
HTEGF16	SEQ ID NO:1818		Cancer
HSUMA53	SEQ ID NO:1819		Cancer
HSUMA53	SEQ ID NO:1820		Cancer
HSUMA53	SEQ ID NO:1821		Cancer
HSUMA53	SEQ ID NO:1822		Cancer
HISSET33	SEQ ID NO:1823		Digestive
HISSET33	SEQ ID NO:1824		Digestive
HTTIJ31	SEQ ID NO:1825		Reproductive
HTTIJ31	SEQ ID NO:1826		Reproductive
HTPFX16	SEQ ID NO:1827		Digestive, Reproductive, Respiratory
HTPFX16	SEQ ID NO:1828		Digestive, Reproductive, Respiratory
HTFMX90	SEQ ID NO:1829		Cancer
HTFMX90	SEQ ID NO:1830		Cancer
HTFMX90	SEQ ID NO:1831		Cancer
HE8FD93	SEQ ID NO:1832		Cancer
HE8FD93	SEQ ID NO:1833		Cancer
HE8FD93	SEQ ID NO:1834		Cancer
HE8FD93	SEQ ID NO:1835		Cancer
HKGBJ74	SEQ ID NO:1836		Cancer
HKGBJ74	SEQ ID NO:1837		Cancer

HKGBJ74	SEQ ID NO:1838	Cancer
HKGBJ74	SEQ ID NO:1839	Cancer
HEEAG84	SEQ ID NO:1840	Reproductive
HEEAG84	SEQ ID NO:1841	Reproductive
HEOQX60	SEQ ID NO:1842	Cancer
HEOQX60	SEQ ID NO:1843	Cancer
HNGGB09	SEQ ID NO:1844	Immune/Hematopoietic
HNGGB09	SEQ ID NO:1845	Immune/Hematopoietic
HKIYI48	SEQ ID NO:1846	Cancer
HKIYI48	SEQ ID NO:1847	Cancer
HKIYI48	SEQ ID NO:1848	Cancer
HKIYI48	SEQ ID NO:1849	Cancer
HSYAB05	SEQ ID NO:1850	Cancer
HSYAB05	SEQ ID NO:1851	Cancer
HARMJ38	SEQ ID NO:1852	Cancer
HARMJ38	SEQ ID NO:1853	Cancer
HARMJ38	SEQ ID NO:1854	Cancer
HARMJ38	SEQ ID NO:1855	Cancer
HDTJG33	SEQ ID NO:1856	Cancer
HWAGJ85	SEQ ID NO:1857	Cardiovascular, Immune/Hematopoietic
HWAGJ85	SEQ ID NO:1858	Cardiovascular, Immune/Hematopoietic
HE2OW03	SEQ ID NO:1859	Mixed Fetal
HE2OW03	SEQ ID NO:1860	Mixed Fetal
HBQAE92	SEQ ID NO:1861	Digestive, Neural/Sensory
HBQAE92	SEQ ID NO:1862	Digestive, Neural/Sensory
HBQAE92	SEQ ID NO:1863	Digestive, Neural/Sensory
HTODL92	SEQ ID NO:1864	Cancer
HTODL92	SEQ ID NO:1865	Cancer
HTODL92	SEQ ID NO:1866	Cancer
HLQBR41	SEQ ID NO:1867	Cancer
HLQBR41	SEQ ID NO:1868	Cancer
HDSAP92	SEQ ID NO:1869	Cancer
HDSAP92	SEQ ID NO:1870	Cancer
HTAEC92	SEQ ID NO:1871	Cancer
HTAEC92	SEQ ID NO:1872	Cancer
HSLCK11	SEQ ID NO:1873	Cancer
HSLCK11	SEQ ID NO:1874	Cancer
HSLCK11	SEQ ID NO:1875	Cancer
HFCDR13	SEQ ID NO:1876	Neural/Sensory
HSLDS06	SEQ ID NO:1877	Musculoskeletal
HSLEF58	SEQ ID NO:1878	Cardiovascular, Digestive, Musculoskeletal
HPCAO10	SEQ ID NO:1879	Cancer
HMEJL61	SEQ ID NO:1880	Cancer
HMEJL61	SEQ ID NO:1881	Cancer
HMEJL61	SEQ ID NO:1882	Cancer
HUSHH92	SEQ ID NO:1883	Cancer
HUSHH92	SEQ ID NO:1884	Cancer
HUSHH92	SEQ ID NO:1885	Cancer
HUSHH92	SEQ ID NO:1886	Cancer



			Reproductive
HNHLA36	SEQ ID NO:1917		Immune/Hematopoietic, Reproductive
HNHOD23	SEQ ID NO:1918		Cancer
HNHOD23	SEQ ID NO:1919		Cancer
HNHOD23	SEQ ID NO:1920		Cancer
HNGNI25	SEQ ID NO:1921		Immune/Hematopoietic
HNGNI25	SEQ ID NO:1922		Immune/Hematopoietic
HNGNI25	SEQ ID NO:1923		Immune/Hematopoietic
HNGNI25	SEQ ID NO:1924		Immune/Hematopoietic
HNGOQ44	SEQ ID NO:1925		Immune/Hematopoietic
HNGOQ44	SEQ ID NO:1926		Immune/Hematopoietic
HTLGE31	SEQ ID NO:1927		Immune/Hematopoietic, Reproductive
HODHE60	SEQ ID NO:1928		Reproductive
HODHE60	SEQ ID NO:1929		Reproductive
HTLIV19	SEQ ID NO:1930		Reproductive
HOSDW58	SEQ ID NO:1931		Cancer
HOSDW58	SEQ ID NO:1932		Cancer
HOSDW58	SEQ ID NO:1933		Cancer
HPJDM47	SEQ ID NO:1934		Reproductive
HPJDM47	SEQ ID NO:1935		Reproductive
HPJEC20	SEQ ID NO:1936		Cancer
HPJEC20	SEQ ID NO:1937		Cancer
HTTJK27	SEQ ID NO:1938		Reproductive
HTTJK27	SEQ ID NO:1939		Reproductive
HTFOE85	SEQ ID NO:1940		Immune/Hematopoietic
HTFOE85	SEQ ID NO:1941		Immune/Hematopoietic
HTFOE85	SEQ ID NO:1942		Immune/Hematopoietic
HIPBA31	SEQ ID NO:1943		Cancer
HIPBA31	SEQ ID NO:1944		Cancer
HFVJY02	SEQ ID NO:1945		Digestive, Mixed Fetal, Neural/Sensory
HFVJY02	SEQ ID NO:1946		Digestive, Mixed Fetal, Neural/Sensory
HFVJY02	SEQ ID NO:1947		Digestive, Mixed Fetal, Neural/Sensory
HFVJY02	SEQ ID NO:1948		Digestive, Mixed Fetal, Neural/Sensory
HFVJY02	SEQ ID NO:1949		Digestive, Mixed Fetal, Neural/Sensory
HOCOO19	SEQ ID NO:1950		Cancer
HOCOO19	SEQ ID NO:1951		Cancer
HOCOO19	SEQ ID NO:1952		Cancer
HWMKQ25	SEQ ID NO:1953		Digestive, Reproductive
HWMKQ25	SEQ ID NO:1954		Digestive, Reproductive
HWMKQ25	SEQ ID NO:1955		Digestive, Reproductive
HCOPG62	SEQ ID NO:1956		Cancer



HCOPG62	SEQ ID NO:1957		Cancer
HNKEL47	SEQ ID NO:1958		Cardiovascular, Connective/Epithelial, Digestive
HNKEL47	SEQ ID NO:1959		Cardiovascular, Connective/Epithelial, Digestive
HTPIY88	SEQ ID NO:1960		Digestive
HTPIY88	SEQ ID NO:1961		Digestive
HTPIY88	SEQ ID NO:1962		Digestive
HTPIY88	SEQ ID NO:1963		Digestive
HEGBS69	SEQ ID NO:1964		Neural/Sensory, Reproductive
HEGBS69	SEQ ID NO:1965		Neural/Sensory, Reproductive
HOFMU07	SEQ ID NO:1966		Reproductive
HOFMU07	SEQ ID NO:1967		Reproductive
HLWBM40	SEQ ID NO:1968		Neural/Sensory, Reproductive
HLWBM40	SEQ ID NO:1969		Neural/Sensory, Reproductive
HLWBM40	SEQ ID NO:1970		Neural/Sensory, Reproductive
HAMFT10	SEQ ID NO:1971		Cancer
HAMFT10	SEQ ID NO:1972		Cancer
HNTBP17	SEQ ID NO:1973		Cancer
HNTBP17	SEQ ID NO:1974		Cancer
HWDAO40	SEQ ID NO:1975		Cancer
HWDAO40	SEQ ID NO:1976		Cancer
HWDAO40	SEQ ID NO:1977		Cancer
HAJCL25	SEQ ID NO:1978		Immune/Hematopoietic
HAJCL25	SEQ ID NO:1979		Immune/Hematopoietic
HAJCL25	SEQ ID NO:1980		Immune/Hematopoietic
HNTEO95	SEQ ID NO:1981		Immune/Hematopoietic
HNTEO95	SEQ ID NO:1982		Immune/Hematopoietic
HNTEO95	SEQ ID NO:1983		Immune/Hematopoietic
HWAFG52	SEQ ID NO:1984		Cancer
HWAFG52	SEQ ID NO:1985		Cancer
HWAFG52	SEQ ID NO:1986		Cancer
HWAFG52	SEQ ID NO:1987		Cancer
HWAHE17	SEQ ID NO:1988		Digestive, Immune/Hematopoietic
HWAHE17	SEQ ID NO:1989		Digestive, Immune/Hematopoietic
HWAHE17	SEQ ID NO:1990		Digestive, Immune/Hematopoietic
HUJBK19	SEQ ID NO:1991		Cancer
HUJBK19	SEQ ID NO:1992		Cancer
HUJBK19	SEQ ID NO:1993		Cancer
HWHJD93	SEQ ID NO:1994		Cancer
HWHJD93	SEQ ID NO:1995		Cancer
HAOST94	SEQ ID NO:1996		Cancer
HAOST94	SEQ ID NO:1997		Cancer
HKZAH22	SEQ ID NO:1998		Reproductive
HKZAH22	SEQ ID NO:1999		Reproductive
HKZAH22	SEQ ID NO:2000		Reproductive

HKZAO35	SEQ ID NO:2001		Reproductive
HKZAO35	SEQ ID NO:2002		Reproductive
HWHSK19	SEQ ID NO:2003		Cancer
HWHSK19	SEQ ID NO:2004		Cancer
HWHSK19	SEQ ID NO:2005		Cancer
HMWFG79	SEQ ID NO:2006		Digestive, Immune/Hematopoietic, Reproductive
HMWFG79	SEQ ID NO:2007		Digestive, Immune/Hematopoietic, Reproductive
HMWFG79	SEQ ID NO:2008		Digestive, Immune/Hematopoietic, Reproductive
HMWFG79	SEQ ID NO:2009		Digestive, Immune/Hematopoietic, Reproductive
HMWFG79	SEQ ID NO:2010		Digestive, Immune/Hematopoietic, Reproductive
HMTAE85	SEQ ID NO:2011		Cancer
HMTBI36	SEQ ID NO:2012		Cancer
HSUME76	SEQ ID NO:2013		Cancer
HSUME76	SEQ ID NO:2014		Cancer
HTEAF65	SEQ ID NO:2015		Excretory, Reproductive
HTEAT31	SEQ ID NO:2016		Cancer
HAJAN23	SEQ ID NO:2017		Cancer
HAPRJ16	SEQ ID NO:2018		Cancer
HDTDT55	SEQ ID NO:2019		Cancer
HSKDA27	SEQ ID NO:2020		Cancer
HSKDA27	SEQ ID NO:2021		Cancer
HWLED11	SEQ ID NO:2022		Cancer
HADGD33	SEQ ID NO:2023		Connective/Epithelial, Neural/Sensory, Reproductive
HCEBF19	SEQ ID NO:2024		Cancer
HCEBF19	SEQ ID NO:2025		Cancer
HDPHH40	SEQ ID NO:2026		Cancer
HHEPM33	SEQ ID NO:2027		Cancer
HJBAF16	SEQ ID NO:2028		Cancer
HJBCU04	SEQ ID NO:2029		Cancer
HWABY10	SEQ ID NO:2030		Cancer
HWABY10	SEQ ID NO:2031		Cancer
HWABY10	SEQ ID NO:2032		Cancer
HWABY10	SEQ ID NO:2033		Cancer
HDPQN11	SEQ ID NO:2034		Cancer
HDPQN11	SEQ ID NO:2035		Cancer
HMSAW68	SEQ ID NO:2036		Cancer
HMSGP80	SEQ ID NO:2037		Cancer
HPJBZ76	SEQ ID NO:2038		Cancer
HSIGM62	SEQ ID NO:2039		Cancer
HSLHS22	SEQ ID NO:2040		Cancer
HTXOZ19	SEQ ID NO:2041		Cancer
HTXOZ19	SEQ ID NO:2042		Cancer
HAPQQ94	SEQ ID NO:2043		Immune/Hematopoietic,

			Reproductive
HAPQQ94	SEQ ID NO:2044		Immune/Hematopoietic, Reproductive
HAPSA79	SEQ ID NO:2045		Cancer
HAPSA79	SEQ ID NO:2046		Cancer
HAPSA79	SEQ ID NO:2047		Cancer
HDP AJ93	SEQ ID NO:2048		Cancer
HELGF34	SEQ ID NO:2049		Cancer
HETEQ88	SEQ ID NO:2050		Cancer
HMSAC18	SEQ ID NO:2051		Cancer
HMSAC18	SEQ ID NO:2052		Cancer
HPQSH59	SEQ ID NO:2053		Cancer
HSIFV30	SEQ ID NO:2054		Cancer
HSVCB08	SEQ ID NO:2055		Cancer
HT3SF53	SEQ ID NO:2056		Cancer
HARMS04	SEQ ID NO:2057		Connective/Epithelial, Digestive
HCDBP36	SEQ ID NO:2058		Musculoskeletal
HCEPE30	SEQ ID NO:2059		Excretory, Neural/Sensory
HE9RM63	SEQ ID NO:2060		Cancer
HKAJF71	SEQ ID NO:2061		Cancer
HNBAF49	SEQ ID NO:2062		Cancer
HSLDJ89	SEQ ID NO:2063		Cancer
HSXGI47	SEQ ID NO:2064		Cancer
HTEAJ18	SEQ ID NO:2065		Reproductive
HTTEV40	SEQ ID NO:2066		Cancer
HWBCB89	SEQ ID NO:2067		Cancer
HWHGZ51	SEQ ID NO:2068		Cancer
HADDH60	SEQ ID NO:2069		Connective/Epithelial, Immune/Hematopoietic, Neural/Sensory
HBXCL93	SEQ ID NO:2070		Neural/Sensory, Reproductive
HPTRH66	SEQ ID NO:2071		Cancer
HNFDH58	SEQ ID NO:2072		Cancer
HACAB58	SEQ ID NO:2073		Cancer
HCE3Z39	SEQ ID NO:2074		Cancer
HCFCU69	SEQ ID NO:2075		Cancer
HCE3Z39	SEQ ID NO:2076		Cancer
HCELE47	SEQ ID NO:2077		Cancer
HCWHP79	SEQ ID NO:2078		Immune/Hematopoietic
HDLAG89	SEQ ID NO:2079		Cancer
HDLAO28	SEQ ID NO:2080		Cancer
HDQGY41	SEQ ID NO:2081		Cancer
HE8FK78	SEQ ID NO:2082		Cancer
HE8FK78	SEQ ID NO:2083		Cancer
HETHR73	SEQ ID NO:2084		Cancer
HFIUW36	SEQ ID NO:2085		Cancer
HFKKS66	SEQ ID NO:2086		Cancer
HFPFK57	SEQ ID NO:2087		Neural/Sensory, Reproductive
HFVJP07	SEQ ID NO:2088		Digestive, Immune/Hematopoietic
HLQEM64	SEQ ID NO:2089		Cancer
HSSDG41	SEQ ID NO:2090		Cancer

HLQGP82	SEQ ID NO:2091		Connective/Epithelial, Digestive, Musculoskeletal
HMSMD07	SEQ ID NO:2092		Cancer
HNGIR58	SEQ ID NO:2093		Immune/Hematopoietic
HMAMI21	SEQ ID NO:2094		Cancer
HNHNB29	SEQ ID NO:2095		Immune/Hematopoietic
HNTEO78	SEQ ID NO:2096		Digestive, Immune/Hematopoietic
HJPAY76	SEQ ID NO:2097		Cancer
HOEEK12	SEQ ID NO:2098		Cancer
HOFNC14	SEQ ID NO:2099		Reproductive
HOSNU69	SEQ ID NO:2100		Cancer
HPJCL28	SEQ ID NO:2101		Neural/Sensory, Reproductive
HRACI26	SEQ ID NO:2102		Digestive, Excretory
HTLIT63	SEQ ID NO:2103		Reproductive
HTEAM34	SEQ ID NO:2104		Reproductive
HTEAM34	SEQ ID NO:2105		Reproductive
HUFGH53	SEQ ID NO:2106		Cancer
HUSBA88	SEQ ID NO:2107		Cancer
HELHN47	SEQ ID NO:2108		Cancer
HELHN47	SEQ ID NO:2109		Cancer
HELHN47	SEQ ID NO:2110		Cancer
HETAY39	SEQ ID NO:2111		Cancer
HFICR14	SEQ ID NO:2112		Cancer
HFICR14	SEQ ID NO:2113		Cancer
HFKET18	SEQ ID NO:2114		Cancer
HFXDK20	SEQ ID NO:2115		Immune/Hematopoietic, Neural/Sensory
HKMLX18	SEQ ID NO:2116		Cancer
HMSCM88	SEQ ID NO:2117		Immune/Hematopoietic
HMABG70	SEQ ID NO:2118		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HMADJ74	SEQ ID NO:2119		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HMADJ14	SEQ ID NO:2120		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HMADJ14	SEQ ID NO:2121		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HMADJ14	SEQ ID NO:2122		Connective/Epithelial, Immune/Hematopoietic, Musculoskeletal
HNEBY54	SEQ ID NO:2123		Cancer
HNEDD37	SEQ ID NO:2124		Cancer
HNGOU82	SEQ ID NO:2125		Immune/Hematopoietic, Reproductive
HNGOW62	SEQ ID NO:2126		Immune/Hematopoietic
HSICO66	SEQ ID NO:2127		Cancer
HSIDQ93	SEQ ID NO:2128		Cancer
HSLGM81	SEQ ID NO:2129		Cancer

HSYBM41	SEQ ID NO:2130	Cancer
HSODB85	SEQ ID NO:2131	Cancer
HSRFZ57	SEQ ID NO:2132	Excretory, Musculoskeletal
HSXAZ05	SEQ ID NO:2133	Neural/Sensory, Respiratory
HTPCW21	SEQ ID NO:2134	Digestive, Neural/Sensory
HTPCW21	SEQ ID NO:2135	Digestive, Neural/Sensory
HTXKF95	SEQ ID NO:2136	Cancer
HTXKF95	SEQ ID NO:2137	Cancer
HUFBC44	SEQ ID NO:2138	Digestive, Mixed Fetal, Neural/Sensory
HAAAI67	SEQ ID NO:2139	Cancer
HFKIA71	SEQ ID NO:2140	Cancer
HAMFP32	SEQ ID NO:2141	Cancer
HAPQU71	SEQ ID NO:2142	Cancer
HAPQU71	SEQ ID NO:2143	Cancer
HLHDL42	SEQ ID NO:2144	Cancer
HAVVG36	SEQ ID NO:2145	Cancer
HBGNP63	SEQ ID NO:2146	Reproductive
HBJNC59	SEQ ID NO:2147	Cancer
HAPQT56	SEQ ID NO:2148	Cancer
HCABW07	SEQ ID NO:2149	Cancer
HDPFB02	SEQ ID NO:2150	Cancer
HMWDB84	SEQ ID NO:2151	Cancer
HDPFB02	SEQ ID NO:2152	Cancer
HDPFY41	SEQ ID NO:2153	Cancer
HDPIE85	SEQ ID NO:2154	Cancer
HDPOE32	SEQ ID NO:2155	Cancer
HWABL61	SEQ ID NO:2156	Cancer
HWABW88	SEQ ID NO:2157	Cancer
HWDAQ83	SEQ ID NO:2158	Cancer
HWDAQ83	SEQ ID NO:2159	Cancer
HWLHZ79	SEQ ID NO:2160	Connective/Epithelial, Digestive, Reproductive
HTXJM94	SEQ ID NO:2161	Cancer
HDPQG01	SEQ ID NO:2162	Cancer
HJPAD80	SEQ ID NO:2163	Cancer
HDPQG01	SEQ ID NO:2164	Cancer
HFXLF67	SEQ ID NO:2165	Neural/Sensory
HE2IO57	SEQ ID NO:2166	Cancer
HKGDP17	SEQ ID NO:2167	Respiratory
HLQFB12	SEQ ID NO:2168	Digestive, Reproductive
HLQFT18	SEQ ID NO:2169	Digestive, Reproductive
HOFNX30	SEQ ID NO:2170	Reproductive
HSSDM23	SEQ ID NO:2171	Cancer
HSSDM23	SEQ ID NO:2172	Cancer
HSVBD67	SEQ ID NO:2173	Cancer
HSVBD67	SEQ ID NO:2174	Cancer
HTGAT51	SEQ ID NO:2175	Cardiovascular,

			Immune/Hematopoietic, Reproductive
HTLGV19	SEQ ID NO:2176		Excretory, Reproductive
HTPHH74	SEQ ID NO:2177		Cancer
HTFOB75	SEQ ID NO:2178		Cancer
HTPHH74	SEQ ID NO:2179		Cancer
HWHGK36	SEQ ID NO:2180		Cancer
HLWAD77	SEQ ID NO:2181		Cancer
HDTGF15	SEQ ID NO:2182		Cancer
HWMBB68	SEQ ID NO:2183		Cancer
HWMBB68	SEQ ID NO:2184		Cancer
HAGDA35	SEQ ID NO:2185		Cancer
HAGDA35	SEQ ID NO:2186		Cancer
HAGDA35	SEQ ID NO:2187		Cancer
HRODQ04	SEQ ID NO:2188		Cancer
HTOJV86	SEQ ID NO:2189		Cancer
HCEFZ82	SEQ ID NO:2190		Cancer
HNGFW58	SEQ ID NO:2191		Cancer
HHBGE77	SEQ ID NO:2192		Cancer
HADFW77	SEQ ID NO:2193		Cancer
HSIED48	SEQ ID NO:2194		Cancer
HCEFZ82	SEQ ID NO:2195		Cancer
HTTCT46	SEQ ID NO:2196		Cancer
HSDEE58	SEQ ID NO:2197		Cancer
HEBCV31	SEQ ID NO:2198		Cancer
HDPOL27	SEQ ID NO:2199		Cancer
HDPOL27	SEQ ID NO:2200		Cancer
HE6DI14	SEQ ID NO:2201		Cancer
HLYAN43	SEQ ID NO:2202		Cancer
HDPUM13	SEQ ID NO:2203		Cancer
HPLAT62	SEQ ID NO:2204		Cancer
HAPQT56	SEQ ID NO:2205		Cancer
HACBG19	SEQ ID NO:2206		Cancer
HACBG19	SEQ ID NO:2207		Cancer
HLYAV34	SEQ ID NO:2208		Cancer
HCNSM85	SEQ ID NO:2209		Cancer
HTOCG60	SEQ ID NO:2210		Cancer
HLYAV34	SEQ ID NO:2211		Cancer
HDPWX42	SEQ ID NO:2212		Cancer
HOFNF53	SEQ ID NO:2213		Reproductive
HOFNF53	SEQ ID NO:2214		Reproductive
HMSEO15	SEQ ID NO:2215		Cancer
HBXFT65	SEQ ID NO:2216		Cancer
HFCEQ37	SEQ ID NO:2217		Cancer
HWNFG66	SEQ ID NO:2218		Digestive
HOHCA60	SEQ ID NO:2219		Cancer
HOHCA60	SEQ ID NO:2220		Cancer
HOHCA60	SEQ ID NO:2221		Cancer
HOHCA60	SEQ ID NO:2222		Cancer
HOHCA60	SEQ ID NO:2223		Cancer
HLDRR08	SEQ ID NO:2224		Digestive
HSKNP59	SEQ ID NO:2225		Musculoskeletal
HSKNP59	SEQ ID NO:2226		Musculoskeletal
HAMHE82	SEQ ID NO:2227		Cancer

HBIOO68	SEQ ID NO:2228	Cancer
HCE3C63	SEQ ID NO:2229	Mixed Fetal, Neural/Sensory
HCNDV12	SEQ ID NO:2230	Digestive, Reproductive
HMWDW68	SEQ ID NO:2231	Cancer
HE2BC57	SEQ ID NO:2232	Cancer
HSDEE58	SEQ ID NO:2233	Cancer
HE9OW91	SEQ ID NO:2234	Cancer
HFCFI20	SEQ ID NO:2235	Cancer
HELEN05	SEQ ID NO:2236	Cancer
HISEL50	SEQ ID NO:2237	Cancer
HLHDL62	SEQ ID NO:2238	Cancer
HDFQB93	SEQ ID NO:2239	Cancer
HLHDQ86	SEQ ID NO:2240	Cancer
HLNAB24	SEQ ID NO:2241	Immune/Hematopoietic
HLYBQ90	SEQ ID NO:2242	Cancer
HLYBQ90	SEQ ID NO:2243	Cancer
HNHDP39	SEQ ID NO:2244	Endocrine, Immune/Hematopoietic, Reproductive
HNTAC64	SEQ ID NO:2245	Cancer
HNTMY29	SEQ ID NO:2246	Connective/Epithelial, Reproductive
HOFOC33	SEQ ID NO:2247	Reproductive
HOFOC33	SEQ ID NO:2248	Reproductive
HTWFK18	SEQ ID NO:2249	Connective/Epithelial, Immune/Hematopoietic
HAPNJ39	SEQ ID NO:2250	Cancer
HDQFU27	SEQ ID NO:2251	Cancer
HETJZ45	SEQ ID NO:2252	Cancer
HTEMX36	SEQ ID NO:2253	Cancer
HNTCH90	SEQ ID NO:2254	Cancer
HWLBP46	SEQ ID NO:2255	Cancer
HA5BM53	SEQ ID NO:2256	Cancer
HMCEH49	SEQ ID NO:2257	Cancer
HKBAL25	SEQ ID NO:2258	Digestive, Musculoskeletal
HE8EF43	SEQ ID NO:2259	Cancer
HE2RN91	SEQ ID NO:2260	Cancer
HTLIO20	SEQ ID NO:2261	Immune/Hematopoietic, Neural/Sensory
HBIMF63	SEQ ID NO:2262	Reproductive
HE9PM90	SEQ ID NO:2263	Cancer
HNTDX22	SEQ ID NO:2264	Reproductive
HHFCE59	SEQ ID NO:2265	Cancer
HCGAD44	SEQ ID NO:2266	Cancer
HSSJJ51	SEQ ID NO:2267	Cancer

In preferred embodiments, the albumin fusion proteins of the invention are capable of a therapeutic activity and/or biologic activity corresponding to the therapeutic activity and/or biologic activity of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein listed in the corresponding row of Table 1.

5 In further preferred embodiments, the therapeutically active protein portions of the albumin fusion proteins of the invention are fragments or variants of the reference sequence cited in the "Exemplary Identifier" column of Table 1, and are capable of the therapeutic activity and/or biologic activity of the corresponding Therapeutic protein.

## 10 ***Polypeptide and Polynucleotide Fragments and Variants***

### *Fragments*

The present invention is further directed to fragments of the Therapeutic proteins described in Table 1, albumin proteins, and/or albumin fusion proteins of the invention.

Even if deletion of one or more amino acids from the N-terminus of a protein results in modification or loss of one or more biological functions of the Therapeutic protein, albumin protein, and/or albumin fusion protein, other Therapeutic activities and/or functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) may still be retained. For example, the ability of polypeptides with N-terminal deletions to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptides generally will be retained when less than the majority of the residues of the complete polypeptide are removed from the N-terminus. Whether a particular polypeptide lacking N-terminal residues of a complete polypeptide retains such immunologic activities can readily be determined by routine methods described herein and otherwise known in the art. It is not unlikely that a mutein with a large number of deleted N-terminal amino acid residues may retain some biological or immunogenic activities. In fact, peptides composed of as few as six amino acid residues may often evoke an immune response.

Accordingly, fragments of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, include the full length protein as well as polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the reference polypeptide (i.e., a Therapeutic protein as disclosed in Table 1). In particular, N-terminal deletions may be described by



the general formula  $m-q$ , where  $q$  is a whole integer representing the total number of amino acid residues in a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1), and  $m$  is defined as any integer ranging from 2 to  $q-6$ . Polynucleotides encoding these polypeptides are also encompassed by the invention.

5 In addition, fragments of serum albumin polypeptides corresponding to an albumin protein portion of an albumin fusion protein of the invention, include the full length protein as well as polypeptides having one or more residues deleted from the amino terminus of the amino acid sequence of the reference polypeptide (i.e., serum albumin). In particular, N-terminal deletions may be described by the general formula  $m-585$ , where  
10 585 is a whole integer representing the total number of amino acid residues in serum albumin (SEQ ID NO:18), and  $m$  is defined as any integer ranging from 2 to 579. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Moreover, fragments of albumin fusion proteins of the invention, include the full length albumin fusion protein as well as polypeptides having one or more residues deleted  
15 from the amino terminus of the albumin fusion protein. In particular, N-terminal deletions may be described by the general formula  $m-q$ , where  $q$  is a whole integer representing the total number of amino acid residues in the albumin fusion protein, and  $m$  is defined as any integer ranging from 2 to  $q-6$ . Polynucleotides encoding these polypeptides are also encompassed by the invention.

20 Also as mentioned above, even if deletion of one or more amino acids from the N-terminus or C-terminus of a reference polypeptide (e.g., a Therapeutic protein and/or serum albumin protein) results in modification or loss of one or more biological functions of the protein, other functional activities (e.g., biological activities, ability to multimerize, ability to bind a ligand) and/or Therapeutic activities may still be retained. For example  
25 the ability of polypeptides with C-terminal deletions to induce and/or bind to antibodies which recognize the complete or mature forms of the polypeptide generally will be retained when less than the majority of the residues of the complete or mature polypeptide are removed from the C-terminus. Whether a particular polypeptide lacking the N-terminal and/or C-terminal residues of a reference polypeptide retains Therapeutic activity  
30 can readily be determined by routine methods described herein and/or otherwise known in the art.

The present invention further provides polypeptides having one or more residues

deleted from the carboxy terminus of the amino acid sequence of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention (e.g., a Therapeutic protein referred to in Table 1). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where q is a whole integer representing the total number of amino acid residues in a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1). Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of the amino acid sequence of an albumin protein corresponding to an albumin protein portion of an albumin fusion protein of the invention (e.g., serum albumin). In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to 584, where 584 is the whole integer representing the total number of amino acid residues in serum albumin (SEQ ID NO:18) minus 1. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Moreover, the present invention provides polypeptides having one or more residues deleted from the carboxy terminus of an albumin fusion protein of the invention. In particular, C-terminal deletions may be described by the general formula 1-n, where n is any whole integer ranging from 6 to q-1, and where q is a whole integer representing the total number of amino acid residues in an albumin fusion protein of the invention. Polynucleotides encoding these polypeptides are also encompassed by the invention.

In addition, any of the above described N- or C-terminal deletions can be combined to produce a N- and C-terminal deleted reference polypeptide. The invention also provides polypeptides having one or more amino acids deleted from both the amino and the carboxyl termini, which may be described generally as having residues m-n of a reference polypeptide (e.g., a Therapeutic protein referred to in Table 1, or serum albumin (e.g., SEQ ID NO:18), or an albumin fusion protein of the invention) where n and m are integers as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

The present application is also directed to proteins containing polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to a reference polypeptide

sequence (e.g., a Therapeutic protein, serum albumin protein or an albumin fusion protein of the invention) set forth herein, or fragments thereof. In preferred embodiments, the application is directed to proteins comprising polypeptides at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to reference polypeptides having the amino acid sequence of N- and C-terminal deletions as described above. Polynucleotides encoding these polypeptides are also encompassed by the invention.

Preferred polypeptide fragments of the invention are fragments comprising, or alternatively, consisting of, an amino acid sequence that displays a Therapeutic activity and/or functional activity (e.g. biological activity) of the polypeptide sequence of the Therapeutic protein or serum albumin protein of which the amino acid sequence is a fragment.

Other preferred polypeptide fragments are biologically active fragments. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of the polypeptide of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

#### *Variants*

"Variant" refers to a polynucleotide or nucleic acid differing from a reference nucleic acid or polypeptide, but retaining essential properties thereof. Generally, variants are overall closely similar, and, in many regions, identical to the reference nucleic acid or polypeptide.

As used herein, "variant", refers to a Therapeutic protein portion of an albumin fusion protein of the invention, albumin portion of an albumin fusion protein of the invention, or albumin fusion protein differing in sequence from a Therapeutic protein (e.g. see "therapeutic" column of Table 1), albumin protein, and/or albumin fusion protein of the invention, respectively, but retaining at least one functional and/or therapeutic property thereof as described elsewhere herein or otherwise known in the art. Generally, variants are overall very similar, and, in many regions, identical to the amino acid sequence of the Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, albumin protein corresponding to an albumin protein portion of an albumin fusion protein of the invention, and/or albumin fusion protein of the invention. Nucleic acids encoding these variants are also encompassed by

the invention.

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The present invention is also directed to proteins which comprise, or alternatively consist of, an amino acid sequence which is at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100%, identical to, for example, the amino acid sequence of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention (e.g., an amino acid sequence disclosed in the "Exemplary Identifier" column of Table 1, or fragments or variants thereof), albumin proteins (e.g., SEQ ID NO:18 or fragments or variants thereof) corresponding to an albumin protein portion of an albumin fusion protein of the invention, and/or albumin fusion proteins of the invention.

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Fragments of these polypeptides are also provided (e.g., those fragments described herein). Further polypeptides encompassed by the invention are polypeptides encoded by polynucleotides which hybridize to the complement of a nucleic acid molecule encoding an amino acid sequence of the invention under stringent hybridization conditions (e.g., hybridization to filter bound DNA in 6X Sodium chloride/Sodium citrate (SSC) at about 45 degrees Celsius, followed by one or more washes in 0.2X SSC, 0.1% SDS at about 50 - 65 degrees Celsius), under highly stringent conditions (e.g., hybridization to filter bound DNA in 6X sodium chloride/Sodium citrate (SSC) at about 45 degrees Celsius, followed by one or more washes in 0.1X SSC, 0.2% SDS at about 68 degrees Celsius), or under other stringent hybridization conditions which are known to those of skill in the art (see, for example, Ausubel, F.M. et al., eds., 1989 *Current protocol in Molecular Biology*, Green publishing associates, Inc., and John Wiley & Sons Inc., New York, at pages 6.3.1 - 6.3.6 and 2.10.3). Polynucleotides encoding these polypeptides are also encompassed by the invention.

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By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to a query amino acid sequence of the present invention, it is intended that the amino acid sequence of the subject polypeptide is identical to the query sequence except that the subject polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a query amino acid sequence, up to 5% of the amino acid residues in the subject sequence may be inserted, deleted, or substituted with another amino acid. These alterations of the reference sequence may occur at the amino- or carboxy-terminal positions of the reference amino

acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

As a practical matter, whether any particular polypeptide is at least 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% identical to, for instance, the amino acid sequence of an albumin fusion protein of the invention or a fragment thereof (such as the Therapeutic protein portion of the albumin fusion protein or the albumin portion of the albumin fusion protein), can be determined conventionally using known computer programs. A preferred method for determining the best overall match between a query sequence (a sequence of the present invention) and a subject sequence, also referred to as a global sequence alignment, can be determined using the FASTDB computer program based on the algorithm of Brutlag et al. (Comp. App. Biosci.6:237-245 (1990)). In a sequence alignment the query and subject sequences are either both nucleotide sequences or both amino acid sequences. The result of said global sequence alignment is expressed as percent identity. Preferred parameters used in a FASTDB amino acid alignment are: Matrix=PAM 0, k-tuple=2, Mismatch Penalty=1, Joining Penalty=20, Randomization Group Length=0, Cutoff Score=1, Window Size=sequence length, Gap Penalty=5, Gap Size Penalty=0.05, Window Size=500 or the length of the subject amino acid sequence, whichever is shorter.

If the subject sequence is shorter than the query sequence due to N- or C-terminal deletions, not because of internal deletions, a manual correction must be made to the results. This is because the FASTDB program does not account for N- and C-terminal truncations of the subject sequence when calculating global percent identity. For subject sequences truncated at the N- and C-termini, relative to the query sequence, the percent identity is corrected by calculating the number of residues of the query sequence that are N- and C-terminal of the subject sequence, which are not matched/aligned with a corresponding subject residue, as a percent of the total bases of the query sequence. Whether a residue is matched/aligned is determined by results of the FASTDB sequence alignment. This percentage is then subtracted from the percent identity, calculated by the above FASTDB program using the specified parameters, to arrive at a final percent identity score. This final percent identity score is what is used for the purposes of the present invention. Only residues to the N- and C-termini of the subject sequence, which

are not matched/aligned with the query sequence, are considered for the purposes of manually adjusting the percent identity score. That is, only query residue positions outside the farthest N- and C- terminal residues of the subject sequence.

For example, a 90 amino acid residue subject sequence is aligned with a 100 residue query sequence to determine percent identity. The deletion occurs at the N-terminus of the subject sequence and therefore, the FASTDB alignment does not show a matching/alignment of the first 10 residues at the N-terminus. The 10 unpaired residues represent 10% of the sequence (number of residues at the N- and C- termini not matched/total number of residues in the query sequence) so 10% is subtracted from the percent identity score calculated by the FASTDB program. If the remaining 90 residues were perfectly matched the final percent identity would be 90%. In another example, a 90 residue subject sequence is compared with a 100 residue query sequence. This time the deletions are internal deletions so there are no residues at the N- or C-termini of the subject sequence which are not matched/aligned with the query. In this case the percent identity calculated by FASTDB is not manually corrected. Once again, only residue positions outside the N- and C-terminal ends of the subject sequence, as displayed in the FASTDB alignment, which are not matched/aligned with the query sequence are manually corrected for. No other manual corrections are to be made for the purposes of the present invention.

The variant will usually have at least 75 % (preferably at least about 80%, 90%, 95% or 99%) sequence identity with a length of normal HA or Therapeutic protein which is the same length as the variant. Homology or identity at the nucleotide or amino acid sequence level is determined by BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, Proc. Natl. Acad. Sci. USA 87: 2264-2268 (1990) and Altschul, J. Mol. Evol. 36: 290-300 (1993), fully incorporated by reference) which are tailored for sequence similarity searching.

The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases, see Altschul *et al.*, (Nature Genetics 6:

119-129 (1994)) which is fully incorporated by reference. The search parameters for histogram, descriptions, alignments, expect (i.e., the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, Proc. Natl. Acad. Sci. USA 89: 10915-10919 (1992), fully incorporated by reference). For blastn, the scoring matrix is set by the ratios of M (i.e., the reward score for a pair of matching residues) to N (i.e., the penalty score for mismatching residues), wherein the default values for M and N are 5 and -4, respectively. Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink<sup>th</sup> position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings were Q=9; R=2; wink=1; and gapw=32. A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

The polynucleotide variants of the invention may contain alterations in the coding regions, non-coding regions, or both. Especially preferred are polynucleotide variants containing alterations which produce silent substitutions, additions, or deletions, but do not alter the properties or activities of the encoded polypeptide. Nucleotide variants produced by silent substitutions due to the degeneracy of the genetic code are preferred. Moreover, polypeptide variants in which less than 50, less than 40, less than 30, less than 20, less than 10, or 5-50, 5-25, 5-10, 1-5, or 1-2 amino acids are substituted, deleted, or added in any combination are also preferred. Polynucleotide variants can be produced for a variety of reasons, e.g., to optimize codon expression for a particular host (change codons in the human mRNA to those preferred by a bacterial host, such as, yeast or *E. coli*).

In a preferred embodiment, a polynucleotide encoding an albumin portion of an albumin fusion protein of the invention is optimized for expression in yeast or mammalian cells. In further preferred embodiment, a polynucleotide encoding a Therapeutic protein portion of an albumin fusion protein of the invention is optimized for expression in yeast or mammalian cells. In a still further preferred embodiment, a polynucleotide encoding an

albumin fusion protein of the invention is optimized for expression in yeast or mammalian cells.

In an alternative embodiment, a codon optimized polynucleotide encoding a Therapeutic protein portion of an albumin fusion protein of the invention does not hybridize to the wild type polynucleotide encoding the Therapeutic protein under stringent hybridization conditions as described herein. In a further embodiment, a codon optimized polynucleotide encoding an albumin portion of an albumin fusion protein of the invention do not hybridize to the wild type polynucleotide encoding the albumin protein under stringent hybridization conditions as described herein. In another embodiment, a codon optimized polynucleotide encoding an albumin fusion protein of the invention do not hybridize to the wild type polynucleotide encoding the Therapeutic protein portion or the albumin protein portion under stringent hybridization conditions as described herein.

In an additional embodiment, polynucleotides encoding a Therapeutic protein portion of an albumin fusion protein of the invention do not comprise, or alternatively consist of, the naturally occurring sequence of that Therapeutic protein. In a further embodiment, polynucleotides encoding an albumin protein portion of an albumin fusion protein of the invention do not comprise, or alternatively consist of, the naturally occurring sequence of albumin protein. In an alternative embodiment, polynucleotides encoding an albumin fusion protein of the invention do not comprise, or alternatively consist of, the naturally occurring sequence of a Therapeutic protein portion or the albumin protein portion.

Naturally occurring variants are called "allelic variants," and refer to one of several alternate forms of a gene occupying a given locus on a chromosome of an organism. (Genes II, Lewin, B., ed., John Wiley & Sons, New York (1985)). These allelic variants can vary at either the polynucleotide and/or polypeptide level and are included in the present invention. Alternatively, non-naturally occurring variants may be produced by mutagenesis techniques or by direct synthesis.

Using known methods of protein engineering and recombinant DNA technology, variants may be generated to improve or alter the characteristics of the polypeptides of the present invention. For instance, one or more amino acids can be deleted from the N-terminus or C-terminus of the polypeptide of the present invention without substantial loss of biological function. As an example, Ron et al. (J. Biol. Chem. 268: 2984-2988 (1993))



reported variant KGF proteins having heparin binding activity even after deleting 3, 8, or 27 amino-terminal amino acid residues. Similarly, Interferon gamma exhibited up to ten times higher activity after deleting 8-10 amino acid residues from the carboxy terminus of this protein. (Dobeli et al., J. Biotechnology 7:199-216 (1988).)

5           Moreover, ample evidence demonstrates that variants often retain a biological activity similar to that of the naturally occurring protein. For example, Gayle and coworkers (J. Biol. Chem. 268:22105-22111 (1993)) conducted extensive mutational analysis of human cytokine IL-1a. They used random mutagenesis to generate over 3,500 individual IL-1a mutants that averaged 2.5 amino acid changes per variant over the entire  
10       length of the molecule. Multiple mutations were examined at every possible amino acid position. The investigators found that "[m]ost of the molecule could be altered with little effect on either [binding or biological activity]." In fact, only 23 unique amino acid sequences, out of more than 3,500 nucleotide sequences examined, produced a protein that significantly differed in activity from wild-type.

15           Furthermore, even if deleting one or more amino acids from the N-terminus or C-terminus of a polypeptide results in modification or loss of one or more biological functions, other biological activities may still be retained. For example, the ability of a deletion variant to induce and/or to bind antibodies which recognize the secreted form will likely be retained when less than the majority of the residues of the secreted form are  
20       removed from the N-terminus or C-terminus. Whether a particular polypeptide lacking N- or C-terminal residues of a protein retains such immunogenic activities can readily be determined by routine methods described herein and otherwise known in the art.

          Thus, the invention further includes polypeptide variants which have a functional activity (e.g., biological activity and/or therapeutic activity). In highly preferred  
25       embodiments the invention provides variants of albumin fusion proteins that have a functional activity (e.g., biological activity and/or therapeutic activity) that corresponds to one or more biological and/or therapeutic activities of the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein. Such variants include deletions, insertions, inversions, repeats, and substitutions selected  
30       according to general rules known in the art so as have little effect on activity.

          In preferred embodiments, the variants of the invention have conservative substitutions. By "conservative substitutions" is intended swaps within groups such as

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replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and  
5 replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly.

Guidance concerning how to make phenotypically silent amino acid substitutions is provided, for example, in Bowie et al., "Deciphering the Message in Protein Sequences: Tolerance to Amino Acid Substitutions," *Science* 247:1306-1310 (1990), wherein the authors indicate that there are two main strategies for studying the tolerance of an amino  
10 acid sequence to change.

The first strategy exploits the tolerance of amino acid substitutions by natural selection during the process of evolution. By comparing amino acid sequences in different species, conserved amino acids can be identified. These conserved amino acids are likely important for protein function. In contrast, the amino acid positions where substitutions  
15 have been tolerated by natural selection indicates that these positions are not critical for protein function. Thus, positions tolerating amino acid substitution could be modified while still maintaining biological activity of the protein.

The second strategy uses genetic engineering to introduce amino acid changes at specific positions of a cloned gene to identify regions critical for protein function. For  
20 example, site directed mutagenesis or alanine-scanning mutagenesis (introduction of single alanine mutations at every residue in the molecule) can be used. See Cunningham and Wells, *Science* 244:1081-1085 (1989). The resulting mutant molecules can then be tested for biological activity.

As the authors state, these two strategies have revealed that proteins are  
25 surprisingly tolerant of amino acid substitutions. The authors further indicate which amino acid changes are likely to be permissive at certain amino acid positions in the protein. For example, most buried (within the tertiary structure of the protein) amino acid residues require nonpolar side chains, whereas few features of surface side chains are generally conserved. Moreover, tolerated conservative amino acid substitutions involve  
30 replacement of the aliphatic or hydrophobic amino acids Ala, Val, Leu and Ile; replacement of the hydroxyl residues Ser and Thr; replacement of the acidic residues Asp and Glu; replacement of the amide residues Asn and Gln, replacement of the basic

residues Lys, Arg, and His; replacement of the aromatic residues Phe, Tyr, and Trp, and replacement of the small-sized amino acids Ala, Ser, Thr, Met, and Gly. Besides conservative amino acid substitution, variants of the present invention include (i) polypeptides containing substitutions of one or more of the non-conserved amino acid residues, where the substituted amino acid residues may or may not be one encoded by the genetic code, or (ii) polypeptides containing substitutions of one or more of the amino acid residues having a substituent group, or (iii) polypeptides which have been fused with or chemically conjugated to another compound, such as a compound to increase the stability and/or solubility of the polypeptide (for example, polyethylene glycol), (iv) polypeptide containing additional amino acids, such as, for example, an IgG Fc fusion region peptide, .

Such variant polypeptides are deemed to be within the scope of those skilled in the art from the teachings herein.

For example, polypeptide variants containing amino acid substitutions of charged amino acids with other charged or neutral amino acids may produce proteins with improved characteristics, such as less aggregation. Aggregation of pharmaceutical formulations both reduces activity and increases clearance due to the aggregate's immunogenic activity. See Pinckard et al., Clin. Exp. Immunol. 2:331-340 (1967); Robbins et al., Diabetes 36: 838-845 (1987); Cleland et al., Crit. Rev. Therapeutic Drug Carrier Systems 10:307-377 (1993).

In specific embodiments, the polypeptides of the invention comprise, or alternatively, consist of, fragments or variants of the amino acid sequence of a Therapeutic protein described herein and/or human serum albumin, and/or albumin fusion protein of the invention, wherein the fragments or variants have 1-5, 5-10, 5-25, 5-50, 10-50 or 50-150, amino acid residue additions, substitutions, and/or deletions when compared to the reference amino acid sequence. In preferred embodiments, the amino acid substitutions are conservative. Nucleic acids encoding these polypeptides are also encompassed by the invention.

The polypeptide of the present invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, i.e., peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. The polypeptides may be modified by either natural processes, such as post-translational processing, or by chemical modification techniques which are well known in the art. Such modifications are

well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in a polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Also, a given polypeptide may contain many types of modifications. Polypeptides may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS - STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

25 *Functional activity*

"A polypeptide having functional activity" refers to a polypeptide capable of displaying one or more known functional activities associated with the full-length, pro-protein, and/or mature form of a Therapeutic protein. Such functional activities include, but are not limited to, biological activity, antigenicity [ability to bind (or compete with a polypeptide for binding) to an anti-polypeptide antibody], immunogenicity (ability to generate antibody which binds to a specific polypeptide of the invention), ability to form multimers with polypeptides of the invention, and ability to bind to a receptor or ligand for

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a polypeptide.

"A polypeptide having biological activity" refers to a polypeptide exhibiting activity similar to, but not necessarily identical to, an activity of a Therapeutic protein of the present invention, including mature forms, as measured in a particular biological assay, with or without dose dependency. In the case where dose dependency does exist, it need not be identical to that of the polypeptide, but rather substantially similar to the dose-dependence in a given activity as compared to the polypeptide of the present invention (i.e., the candidate polypeptide will exhibit greater activity or not more than about 25-fold less and, preferably, not more than about tenfold less activity, and most preferably, not more than about three-fold less activity relative to the polypeptide of the present invention).

In preferred embodiments, an albumin fusion protein of the invention has at least one biological and/or therapeutic activity associated with the Therapeutic protein (or fragment or variant thereof) when it is not fused to albumin.

The albumin fusion proteins of the invention can be assayed for functional activity (e.g., biological activity) using or routinely modifying assays known in the art, as well as assays described herein. Additionally, one of skill in the art may routinely assay fragments of a Therapeutic protein corresponding to a Therapeutic protein portion of an albumin fusion protein of the invention, for activity using assays referenced in its corresponding row of Table 1. Further, one of skill in the art may routinely assay fragments of an albumin protein corresponding to an albumin protein portion of an albumin fusion protein of the invention, for activity using assays known in the art and/or as described in the Examples section below.

For example, in one embodiment where one is assaying for the ability of an albumin fusion protein of the invention to bind or compete with a Therapeutic protein for binding to an anti-Therapeutic polypeptide antibody and/or anti-albumin antibody, various immunoassays known in the art can be used, including but not limited to, competitive and non-competitive assay systems using techniques such as radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoradiometric assays, gel diffusion precipitation reactions, immunodiffusion assays, in situ immunoassays (using colloidal gold, enzyme or radioisotope labels, for example), western blots, precipitation reactions, agglutination assays (e.g., gel agglutination assays,

hemagglutination assays), complement fixation assays, immunofluorescence assays, protein A assays, and immunoelectrophoresis assays, etc. In one embodiment, antibody binding is detected by detecting a label on the primary antibody. In another embodiment, the primary antibody is detected by detecting binding of a secondary antibody or reagent to the primary antibody. In a further embodiment, the secondary antibody is labeled. Many means are known in the art for detecting binding in an immunoassay and are within the scope of the present invention.

In a preferred embodiment, where a binding partner (e.g., a receptor or a ligand) of a Therapeutic protein is identified, binding to that binding partner by an albumin fusion protein containing that Therapeutic protein as the Therapeutic protein portion of the fusion can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., *Microbiol. Rev.* 59:94-123 (1995). In another embodiment, the ability of physiological correlates of an albumin fusion protein of the present invention to bind to a substrate(s) of the Therapeutic polypeptide corresponding to the Therapeutic portion of the albumin fusion protein of the invention can be routinely assayed using techniques known in the art.

In an alternative embodiment, where the ability of an albumin fusion protein of the invention to multimerize is being evaluated, association with other components of the multimer can be assayed, e.g., by means well-known in the art, such as, for example, reducing and non-reducing gel chromatography, protein affinity chromatography, and affinity blotting. See generally, Phizicky et al., *supra*.

In preferred embodiments, an albumin fusion protein of the invention comprising all or a portion of an antibody that binds a Therapeutic protein, has at least one biological and/or therapeutic activity (e.g., to specifically bind a polypeptide or epitope) associated with the antibody that binds a Therapeutic protein (or fragment or variant thereof) when it is not fused to albumin. In other preferred embodiments, the biological activity and/or therapeutic activity of an albumin fusion protein of the invention comprising all or a portion of an antibody that binds a Therapeutic protein is the inhibition (i.e. antagonism) or activation (i.e., agonism) of one or more of the biological activities and/or therapeutic activities associated with the polypeptide that is specifically bound by antibody that binds a Therapeutic protein.

Albumin fusion proteins of the invention (e.g., comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) may be characterized in a variety of ways. In particular, albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for the ability to specifically bind to the same antigens specifically bound by the antibody that binds a Therapeutic protein corresponding to the Therapeutic protein portion of the albumin fusion protein using techniques described herein or routinely modifying techniques known in the art.

Assays for the ability of the albumin fusion proteins of the invention (e.g., comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to (specifically) bind a specific protein or epitope may be performed in solution (e.g., Houghten, *Bio/Techniques* 13:412-421(1992)), on beads (e.g., Lam, *Nature* 354:82-84 (1991)), on chips (e.g., Fodor, *Nature* 364:555-556 (1993)), on bacteria (e.g., U.S. Patent No. 5,223,409), on spores (e.g., Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (e.g., Cull et al., *Proc. Natl. Acad. Sci. USA* 89:1865-1869 (1992)) or on phage (e.g., Scott and Smith, *Science* 249:386-390 (1990); Devlin, *Science* 249:404-406 (1990); Cwirla et al., *Proc. Natl. Acad. Sci. USA* 87:6378-6382 (1990); and Felici, *J. Mol. Biol.* 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). Albumin fusion proteins of the invention comprising at least a fragment or variant of a Therapeutic antibody may also be assayed for their specificity and affinity for a specific protein or epitope using or routinely modifying techniques described herein or otherwise known in the art.

The albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for cross-reactivity with other antigens (e.g., molecules that have sequence/structure conservation with the molecule(s) specifically bound by the antibody that binds a Therapeutic protein (or fragment or variant thereof) corresponding to the Therapeutic protein portion of the albumin fusion protein of the invention) by any method known in the art.

Immunoassays which can be used to analyze (immunospecific) binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin

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reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (*e.g.*, EDTA, PMSF, aprotinin, sodium vanadate), adding the albumin fusion protein of the invention (*e.g.*, comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to the cell lysate, incubating for a period of time (*e.g.*, 1 to 4 hours) at 40 degrees C, adding sepharose beads coupled to an anti-albumin antibody, for example, to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the albumin fusion protein of the invention to immunoprecipitate a particular antigen can be assessed by, *e.g.*, western blot analysis. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the binding of the albumin fusion protein to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), applying the albumin fusion protein of the invention (diluted in blocking buffer) to the membrane, washing the membrane in washing buffer, applying a secondary antibody (which recognizes the albumin fusion protein, *e.g.*, an anti-human serum albumin antibody) conjugated to an enzymatic substrate



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(*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the albumin fusion protein (*e.g.*, comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) of the invention conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound or non-specifically bound albumin fusion proteins, and detecting the presence of the albumin fusion proteins specifically bound to the antigen coating the well. In ELISAs the albumin fusion protein does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes albumin fusion protein) conjugated to a detectable compound may be added to the well. Further, instead of coating the well with the antigen, the albumin fusion protein may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an albumin fusion protein to a protein, antigen, or epitope and the off-rate of an albumin fusion protein-protein/antigen/epitope interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the albumin fusion protein of the invention in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the albumin fusion protein of the present invention for a specific protein, antigen, or epitope

and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second protein that binds the same protein, antigen or epitope as the albumin fusion protein, can also be determined using radioimmunoassays. In this case, the protein, antigen or epitope is incubated with an albumin fusion protein of the present invention conjugated to a labeled compound (e.g.,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second protein that binds the same protein, antigen, or epitope as the albumin fusion protein of the invention.

In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of albumin fusion proteins of the invention to a protein, antigen or epitope. BIAcore kinetic analysis comprises analyzing the binding and dissociation of albumin fusion proteins, or specific polypeptides, antigens or epitopes from chips with immobilized specific polypeptides, antigens or epitopes or albumin fusion proteins, respectively, on their surface.

Antibodies that bind a Therapeutic protein corresponding to the Therapeutic protein portion of an albumin fusion protein of the invention may also be described or specified in terms of their binding affinity for a given protein or antigen, preferably the antigen which they specifically bind. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M. More preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M or  $10^{-8}$  M.

Even more preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has an affinity for a given protein or epitope similar to that of the corresponding antibody (not fused to albumin) that binds a Therapeutic protein, taking into account the valency of the albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) and the valency of the corresponding antibody. In addition, assays described herein (see Examples and Table 1) and otherwise known in the art may routinely be applied to measure the ability of albumin fusion proteins of the present invention and fragments, variants and derivatives thereof to elicit biological activity and/or Therapeutic activity

(either *in vitro* or *in vivo*) related to either the Therapeutic protein portion and/or albumin portion of the albumin fusion protein of the present invention. Other methods will be known to the skilled artisan and are within the scope of the invention.

## 5           **Albumin**

As described above, an albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion or chemical conjugation.

10           The terms, human serum albumin (HSA) and human albumin (HA) are used interchangeably herein. The terms, "albumin and "serum albumin" are broader, and encompass human serum albumin (and fragments and variants thereof) as well as albumin from other species (and fragments and variants thereof).

As used herein, "albumin" refers collectively to albumin protein or amino acid  
15           sequence, or an albumin fragment or variant, having one or more functional activities (e.g., biological activities) of albumin. In particular, "albumin" refers to human albumin or fragments thereof (see EP 201 239, EP 322 094 WO 97/24445, WO95/23857) especially the mature form of human albumin as shown in Figure 15 and SEQ ID NO:18, or albumin from other vertebrates or fragments thereof, or analogs or variants of these molecules or  
20           fragments thereof.

In preferred embodiments, the human serum albumin protein used in the albumin fusion proteins of the invention contains one or both of the following sets of point mutations with reference to SEQ ID NO:18: Leu-407 to Ala, Leu-408 to Val, Val-409 to Ala, and Arg-410 to Ala; or Arg-410 to A, Lys-413 to Gln, and Lys-414 to Gln (see, e.g.,  
25           International Publication No. WO95/23857, hereby incorporated in its entirety by reference herein). In even more preferred embodiments, albumin fusion proteins of the invention that contain one or both of above-described sets of point mutations have improved stability/resistance to yeast Yap3p proteolytic cleavage, allowing increased production of recombinant albumin fusion proteins expressed in yeast host cells.

30           As used herein, a portion of albumin sufficient to prolong the therapeutic activity or shelf-life of the Therapeutic protein refers to a portion of albumin sufficient in length or structure to stabilize or prolong the therapeutic activity of the protein so that the shelf life

of the Therapeutic protein portion of the albumin fusion protein is prolonged or extended compared to the shelf-life in the non-fusion state. The albumin portion of the albumin fusion proteins may comprise the full length of the HA sequence as described above or as shown in Figure 15, or may include one or more fragments thereof that are capable of stabilizing or prolonging the therapeutic activity. Such fragments may be of 10 or more amino acids in length or may include about 15, 20, 25, 30, 50, or more contiguous amino acids from the HA sequence or may include part or all of specific domains of HA. For instance, one or more fragments of HA spanning the first two immunoglobulin-like domains may be used.

The albumin portion of the albumin fusion proteins of the invention may be a variant of normal HA. The Therapeutic protein portion of the albumin fusion proteins of the invention may also be variants of the Therapeutic proteins as described herein. The term "variants" includes insertions, deletions and substitutions, either conservative or non-conservative, where such changes do not substantially alter one or more of the oncotic, useful ligand-binding and non-immunogenic properties of albumin, or the active site, or active domain which confers the therapeutic activities of the Therapeutic proteins.

In particular, the albumin fusion proteins of the invention may include naturally occurring polymorphic variants of human albumin and fragments of human albumin, for example those fragments disclosed in EP 322 094 (namely HA (Pn), where n is 369 to 419). The albumin may be derived from any vertebrate, especially any mammal, for example human, cow, sheep, or pig. Non-mammalian albumins include, but are not limited to, hen and salmon. The albumin portion of the albumin fusion protein may be from a different animal than the Therapeutic protein portion.

Generally speaking, an HA fragment or variant will be at least 100 amino acids long, preferably at least 150 amino acids long. The HA variant may consist of or alternatively comprise at least one whole domain of HA, for example domains 1 (amino acids 1-194 of SEQ ID NO:18), 2 (amino acids 195-387 of SEQ ID NO:18), 3 (amino acids 388-585 of SEQ ID NO:18), 1 + 2 (1-387 of SEQ ID NO:18), 2 + 3 (195-585 of SEQ ID NO:18) or 1 + 3 (amino acids 1-194 of SEQ ID NO:18 + amino acids 388-585 of SEQ ID NO:18). Each domain is itself made up of two homologous subdomains namely 1-105, 120-194, 195-291, 316-387, 388-491 and 512-585, with flexible inter-subdomain linker regions comprising residues Lys106 to Glu119, Glu292 to Val315 and Glu492 to

Ala511.

Preferably, the albumin portion of an albumin fusion protein of the invention comprises at least one subdomain or domain of HA or conservative modifications thereof.

If the fusion is based on subdomains, some or all of the adjacent linker is preferably used  
5 to link to the Therapeutic protein moiety.

*Antibodies that Specifically bind Therapeutic proteins are also Therapeutic proteins*

The present invention also encompasses albumin fusion proteins that comprise at  
10 least a fragment or variant of an antibody that specifically binds a Therapeutic protein  
disclosed in Table 1. It is specifically contemplated that the term "Therapeutic protein"  
encompasses antibodies that bind a Therapeutic protein (e.g., as Described in column I of  
Table 1) and fragments and variants thereof. Thus an albumin fusion protein of the  
invention may contain at least a fragment or variant of a Therapeutic protein, and/or at  
15 least a fragment or variant of an an antibody that binds a Therapeutic protein.

*Antibody structure and background*

The basic antibody structural unit is known to comprise a tetramer. Each tetramer is  
20 composed of two identical pairs of polypeptide chains, each pair having one "light" (about  
25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each  
chain includes a variable region of about 100 to 110 or more amino acids primarily  
responsible for antigen recognition. The carboxy-terminal portion of each chain defines a  
constant region primarily responsible for effector function. Human light chains are  
25 classified as kappa and lambda light chains. Heavy chains are classified as mu, delta,  
gamma, alpha, or epsilon, and define the antibody's isotype as IgM, IgD, IgG, IgA, and  
IgE, respectively. *See generally, Fundamental Immunology* Chapters 3-5 (Paul, W., ed.,  
4th ed. Raven Press, N.Y. (1998)) (incorporated by reference in its entirety for all  
purposes). The variable regions of each light/heavy chain pair form the antibody binding  
30 site.

Thus, an intact IgG antibody has two binding sites. Except in bifunctional or  
bispecific antibodies, the two binding sites are the same.

FOI b7E b7C b7D b7F b7G b7H b7I b7J b7K b7L b7M b7N b7O b7P b7Q b7R b7S b7T b7U b7V b7W b7X b7Y b7Z b7AA b7AB b7AC b7AD b7AE b7AF b7AG b7AH b7AI b7AJ b7AK b7AL b7AM b7AN b7AO b7AP b7AQ b7AR b7AS b7AT b7AU b7AV b7AW b7AX b7AY b7AZ b7BA b7BB b7BC b7BD b7BE b7BF b7BG b7BH b7BI b7BJ b7BK b7BL b7BM b7BN b7BO b7BP b7BQ b7BR b7BS b7BT b7BU b7BV b7BW b7BX b7BY b7BZ b7CA b7CB b7CC b7CD b7CE b7CF b7CG b7CH b7CI b7CJ b7CK b7CL b7CM b7CN b7CO b7CP b7CQ b7CR b7CS b7CT b7CU b7CV b7CW b7CX b7CY b7CZ b7DA b7DB b7DC b7DD b7DE b7DF b7DG b7DH b7DI b7DJ b7DK b7DL b7DM b7DN b7DO b7DP b7DQ b7DR b7DS b7DT b7DU b7DV b7DW b7DX b7DY b7DZ b7EA b7EB b7EC b7ED b7EE b7EF b7EG b7EH b7EI b7EJ b7EK b7EL b7EM b7EN b7EO b7EP b7EQ b7ER b7ES b7ET b7EU b7EV b7EW b7EX b7EY b7EZ b7FA b7FB b7FC b7FD b7FE b7FF b7FG b7FH b7FI b7FJ b7FK b7FL b7FM b7FN b7FO b7FP b7FQ b7FR b7FS b7FT b7FU b7FV b7FW b7FX b7FY b7FZ b7GA b7GB b7GC b7GD b7GE b7GF b7GG b7GH b7GI b7GJ b7GK b7GL b7GM b7GN b7GO b7GP b7GQ b7GR b7GS b7GT b7GU b7GV b7GW b7GX b7GY b7GZ b7HA b7HB b7HC b7HD b7HE b7HF b7HG b7HH b7HI b7HJ b7HK b7HL b7HM b7HN b7HO b7HP b7HQ b7HR b7HS b7HT b7HU b7HV b7HW b7HX b7HY b7HZ b7IA b7IB b7IC b7ID b7IE b7IF b7IG b7IH b7IJ b7IK b7IL b7IM b7IN b7IO b7IP b7IQ b7IR b7IS b7IT b7IU b7IV b7IW b7IX b7IY b7IZ b7JA b7JB b7JC b7JD b7JE b7JF b7JG b7JH b7JI b7JJ b7JK b7JL b7JM b7JN b7JO b7JP b7JQ b7JR b7JS b7JT b7JU b7JV b7JW b7JX b7JY b7JZ b7KA b7KB b7KC b7KD b7KE b7KF b7KG b7KH b7KI b7KJ b7KK b7KL b7KM b7KN b7KO b7KP b7KQ b7KR b7KS b7KT b7KU b7KV b7KW b7KX b7KY b7KZ b7LA b7LB b7LC b7LD b7LE b7LF b7LG b7LH b7LI b7LJ b7LK b7LL b7LM b7LN b7LO b7LP b7LQ b7LR b7LS b7LT b7LU b7LV b7LW b7LX b7LY b7LZ b7MA b7MB b7MC b7MD b7ME b7MF b7MG b7MH b7MI b7MJ b7MK b7ML b7MN b7MO b7MP b7MQ b7MR b7MS b7MT b7MU b7MV b7MW b7MX b7MY b7MZ b7NA b7NB b7NC b7ND b7NE b7NF b7NG b7NH b7NI b7NJ b7NK b7NL b7NM b7NO b7NP b7NQ b7NR b7NS b7NT b7NU b7NV b7NW b7NX b7NY b7NZ b7OA b7OB b7OC b7OD b7OE b7OF b7OG b7OH b7OI b7OJ b7OK b7OL b7OM b7ON b7OO b7OP b7OQ b7OR b7OS b7OT b7OU b7OV b7OW b7OX b7OY b7OZ b7PA b7PB b7PC b7PD b7PE b7PF b7PG b7PH b7PI b7PJ b7PK b7PL b7PM b7PN b7PO b7PP b7PQ b7PR b7PS b7PT b7PU b7PV b7PW b7PX b7PY b7PZ b7QA b7QB b7QC b7QD b7QE b7QF b7QG b7QH b7QI b7QJ b7QK b7QL b7QM b7QN b7QO b7QP b7QQ b7QR b7QS b7QT b7QU b7QV b7QW b7QX b7QY b7QZ b7RA b7RB b7RC b7RD b7RE b7RF b7RG b7RH b7RI b7RJ b7RK b7RL b7RM b7RN b7RO b7RP b7RQ b7RR b7RS b7RT b7RU b7RV b7RW b7RX b7RY b7RZ b7SA b7SB b7SC b7SD b7SE b7SF b7SG b7SH b7SI b7SJ b7SK b7SL b7SM b7SN b7SO b7SP b7SQ b7SR b7SS b7ST b7SU b7SV b7SW b7SX b7SY b7SZ b7TA b7TB b7TC b7TD b7TE b7TF b7TG b7TH b7TI b7TJ b7TK b7TL b7TM b7TN b7TO b7TP b7TQ b7TR b7TS b7TT b7TU b7TV b7TW b7TX b7TY b7TZ b7UA b7UB b7UC b7UD b7UE b7UF b7UG b7UH b7UI b7UJ b7UK b7UL b7UM b7UN b7UO b7UP b7UQ b7UR b7US b7UT b7UU b7UV b7UW b7UX b7UY b7UZ b7VA b7VB b7VC b7VD b7VE b7VF b7VG b7VH b7VI b7VJ b7VK b7VL b7VM b7VN b7VO b7VP b7VQ b7VR b7VS b7VT b7VU b7VV b7VW b7VX b7VY b7VZ b7WA b7WB b7WC b7WD b7WE b7WF b7WG b7WH b7WI b7WJ b7WK b7WL b7WM b7WN b7WO b7WP b7WQ b7WR b7WS b7WT b7WU b7WV b7WW b7WX b7WY b7WZ b7XA b7XB b7XC b7XD b7XE b7XF b7XG b7XH b7XI b7XJ b7XK b7XL b7XM b7XN b7XO b7XP b7XQ b7XR b7XS b7XT b7XU b7XV b7XW b7XX b7XY b7XZ b7YA b7YB b7YC b7YD b7YE b7YF b7YG b7YH b7YI b7YJ b7YK b7YL b7YM b7YN b7YO b7YP b7YQ b7YR b7YS b7YT b7YU b7YV b7YW b7YX b7YY b7YZ b7ZA b7ZB b7ZC b7ZD b7ZE b7ZF b7ZG b7ZH b7ZI b7ZJ b7ZK b7ZL b7ZM b7ZN b7ZO b7ZP b7ZQ b7ZR b7ZS b7ZT b7ZU b7ZV b7ZW b7ZX b7ZY b7ZZ b7AA b7AB b7AC b7AD b7AE b7AF b7AG b7AH b7AI b7AJ b7AK b7AL b7AM b7AN b7AO b7AP b7AQ b7AR b7AS b7AT b7AU b7AV b7AW b7AX b7AY b7AZ b7BA b7BB b7BC b7BD b7BE b7BF b7BG b7BH b7BI b7BJ b7BK b7BL b7BM b7BN b7BO b7BP b7BQ b7BR b7BS b7BT b7BU b7BV b7BW b7BX b7BY b7BZ b7CA b7CB b7CC b7CD b7CE b7CF b7CG b7CH b7CI b7CJ b7CK b7CL b7CM b7CN b7CO b7CP b7CQ b7CR b7CS b7CT b7CU b7CV b7CW b7CX b7CY b7CZ b7DA b7DB b7DC b7DD b7DE b7DF b7DG b7DH b7DI b7DJ b7DK b7DL b7DM b7DN b7DO b7DP b7DQ b7DR b7DS b7DT b7DU b7DV b7DW b7DX b7DY b7DZ b7EA b7EB b7EC b7ED b7EE b7EF b7EG b7EH b7EI b7EJ b7EK b7EL b7EM b7EN b7EO b7EP b7EQ b7ER b7ES b7ET b7EU b7EV b7EW b7EX b7EY b7EZ b7FA b7FB b7FC b7FD b7FE b7FF b7FG b7FH b7FI b7FJ b7FK b7FL b7FM b7FN b7FO b7FP b7FQ b7FR b7FS b7FT b7FU b7FV b7FW b7FX b7FY b7FZ b7GA b7GB b7GC b7GD b7GE b7GF b7GG b7GH b7GI b7GJ b7GK b7GL b7GM b7GN b7GO b7GP b7GQ b7GR b7GS b7GT b7GU b7GV b7GW b7GX b7GY b7GZ b7HA b7HB b7HC b7HD b7HE b7HF b7HG b7HH b7HI b7HJ b7HK b7HL b7HM b7HN b7HO b7HP b7HQ b7HR b7HS b7HT b7HU b7HV b7HW b7HX b7HY b7HZ b7IA b7IB b7IC b7ID b7IE b7IF b7IG b7IH b7IJ b7IK b7IL b7IM b7IN b7IO b7IP b7IQ b7IR b7IS b7IT b7IU b7IV b7IW b7IX b7IY b7IZ b7JA b7JB b7JC b7JD b7JE b7JF b7JG b7JH b7JI b7JJ b7JK b7JL b7JM b7JN b7JO b7JP b7JQ b7JR b7JS b7JT b7JU b7JV b7JW b7JX b7JY b7JZ b7KA b7KB b7KC b7KD b7KE b7KF b7KG b7KH b7KI b7KJ b7KK b7KL b7KM b7KN b7KO b7KP b7KQ b7KR b7KS b7KT b7KU b7KV b7KW b7KX b7KY b7KZ b7LA b7LB b7LC b7LD b7LE b7LF b7LG b7LH b7LI b7LJ b7LK b7LL b7LM b7LN b7LO b7LP b7LQ b7LR b7LS b7LT b7LU b7LV b7LW b7LX b7LY b7LZ b7MA b7MB b7MC b7MD b7ME b7MF b7MG b7MH b7MI b7MJ b7MK b7ML b7MN b7MO b7MP b7MQ b7MR b7MS b7MT b7MU b7MV b7MW b7MX b7MY b7MZ b7NA b7NB b7NC b7ND b7NE b7NF b7NG b7NH b7NI b7NJ b7NK b7NL b7NM b7NO b7NP b7NQ b7NR b7NS b7NT b7NU b7NV b7NW b7NX b7NY b7NZ b7OA b7OB b7OC b7OD b7OE b7OF b7OG b7OH b7OI b7OJ b7OK b7OL b7OM b7ON b7OO b7OP b7OQ b7OR b7OS b7OT b7OU b7OV b7OW b7OX b7OY b7OZ b7PA b7PB b7PC b7PD b7PE b7PF b7PG b7PH b7PI b7PJ b7PK b7PL b7PM b7PN b7PO b7PP b7PQ b7PR b7PS b7PT b7PU b7PV b7PW b7PX b7PY b7PZ b7QA b7QB b7QC b7QD b7QE b7QF b7QG b7QH b7QI b7QJ b7QK b7QL b7QM b7QN b7QO b7QP b7QQ b7QR b7QS b7QT b7QU b7QV b7QW b7QX b7QY b7QZ b7RA b7RB b7RC b7RD b7RE b7RF b7RG b7RH b7RI b7RJ b7RK b7RL b7RM b7RN b7RO b7RP b7RQ b7RR b7RS b7RT b7RU b7RV b7RW b7RX b7RY b7RZ b7SA b7SB b7SC b7SD b7SE b7SF b7SG b7SH b7SI b7SJ b7SK b7SL b7SM b7SN b7SO b7SP b7SQ b7SR b7SS b7ST b7SU b7SV b7SW b7SX b7SY b7SZ b7TA b7TB b7TC b7TD b7TE b7TF b7TG b7TH b7TI b7TJ b7TK b7TL b7TM b7TN b7TO b7TP b7TQ b7TR b7TS b7TT b7TU b7TV b7TW b7TX b7TY b7TZ b7UA b7UB b7UC b7UD b7UE b7UF b7UG b7UH b7UI b7UJ b7UK b7UL b7UM b7UN b7UO b7UP b7UQ b7UR b7US b7UT b7UU b7UV b7UW b7UX b7UY b7UZ b7VA b7VB b7VC b7VD b7VE b7VF b7VG b7VH b7VI b7VJ b7VK b7VL b7VM b7VN b7VO b7VP b7VQ b7VR b7VS b7VT b7VU b7VV b7VW b7VX b7VY b7VZ b7WA b7WB b7WC b7WD b7WE b7WF b7WG b7WH b7WI b7WJ b7WK b7WL b7WM b7WN b7WO b7WP b7WQ b7WR b7WS b7WT b7WU b7WV b7WW b7WX b7WY b7WZ b7XA b7XB b7XC b7XD b7XE b7XF b7XG b7XH b7XI b7XJ b7XK b7XL b7XM b7XN b7XO b7XP b7XQ b7XR b7XS b7XT b7XU b7XV b7XW b7XX b7XY b7XZ b7YA b7YB b7YC b7YD b7YE b7YF b7YG b7YH b7YI b7YJ b7YK b7YL b7YM b7YN b7YO b7YP b7YQ b7YR b7YS b7YT b7YU b7YV b7YW b7YX b7YY b7YZ b7ZA b7ZB b7ZC b7ZD b7ZE b7ZF b7ZG b7ZH b7ZI b7ZJ b7ZK b7ZL b7ZM b7ZN b7ZO b7ZP b7ZQ b7ZR b7ZS b7ZT b7ZU b7ZV b7ZW b7ZX b7ZY b7ZZ

The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDR regions, in general, are the portions of the antibody which make contact with the antigen and determine its specificity. The CDRs from the heavy and the light chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains variable regions comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The variable regions are connected to the heavy or light chain constant region. The assignment of amino acids to each domain is in accordance with the definitions of Kabat *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, Md. (1987 and 1991)), or Chothia & Lesk *J Mol. Biol.* 196:901-917 (1987); Chothia et al. *Nature* 342:878-883 (1989).

As used herein, "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that specifically binds an antigen (e.g., a molecule containing one or more CDR regions of an antibody). Antibodies that may correspond to a Therapeutic protein portion of an albumin fusion protein include, but are not limited to, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies (e.g., single chain Fvs), Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies specific to antibodies of the invention), and epitope-binding fragments of any of the above (e.g., VH domains, VL domains, or one or more CDR regions).

#### *Antibodies that bind Therapeutic Proteins*

The present invention encompasses albumin fusion proteins that comprise at least a fragment or variant of an antibody that binds a Therapeutic Protein (e.g., as disclosed in Table 1) or fragment or variant thereof.

Antibodies that bind a Therapeutic protein (or fragment or variant thereof) may be from any animal origin, including birds and mammals. Preferably, the antibodies are human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken antibodies. Most preferably, the antibodies are human antibodies. As used herein, "human" antibodies include antibodies having the amino acid sequence of a human

immunoglobulin and include antibodies isolated from human immunoglobulin libraries and xenomice or other organisms that have been genetically engineered to produce human antibodies.

The antibody molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule. In preferred embodiments, the antibody molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention are IgG1. In other preferred embodiments, the immunoglobulin molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention are IgG2. In other preferred embodiments, the immunoglobulin molecules that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention are IgG4.

Most preferably the antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention are human antigen-binding antibody fragments of the present invention and include, but are not limited to, Fab, Fab' and F(ab')<sub>2</sub>, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a VL or VH domain. Antigen-binding antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains.

The antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be monospecific, bispecific, trispecific or of greater multispecificity. Multispecific antibodies may be specific for different epitopes of a Therapeutic protein or may be specific for both a Therapeutic protein as well as for a heterologous epitope, such as a heterologous polypeptide or solid support material. See, e.g., PCT publications WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, et al., J. Immunol. 147:60-69 (1991); U.S. Patent Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; Kostelny et al., J. Immunol. 148:1547-1553 (1992).

Antibodies that bind a Therapeutic protein (or fragment or variant thereof) may be

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bispecific or bifunctional which means that the antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann *Clin. Exp. Immunol.* 79: 315-321 (1990), Kostelny et al. *J Immunol.* 148:1547-1553 (1992). In addition, bispecific antibodies may be formed as "diabodies" (Holliger et al. "Diabodies: small bivalent and bispecific antibody fragments" *PNAS USA* 90:6444-6448 (1993)) or "Janusins" (Traunecker et al. "Bispecific single chain molecules (Janusins) target cytotoxic lymphocytes on HIV infected cells" *EMBO J* 10:3655-3659 (1991) and Traunecker et al. "Janusin: new molecular design for bispecific reagents" *Int J Cancer Suppl* 7:51-52 (1992)).

The present invention also provides albumin fusion proteins that comprise, fragments or variants (including derivatives) of an antibody described herein or known elsewhere in the art. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding a molecule of the invention, including, for example, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5 amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH domain, VHCDR1, VHCDR2, VHCDR3, VL domain, VLCDR1, VLCDR2, or VLCDR3. In specific embodiments, the variants encode substitutions of VHCDR3. In a preferred embodiment, the variants have conservative amino acid substitutions at one or more predicted non-essential amino acid residues.

Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be described or specified in terms of the epitope(s) or portion(s) of a Therapeutic protein which they recognize or specifically bind. Antibodies which specifically bind a Therapeutic protein or a specific epitope of a Therapeutic protein may also be excluded. Therefore, the present invention encompasses antibodies that specifically bind Therapeutic



proteins, and allows for the exclusion of the same. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, binds the same epitopes as the .

Antibodies that bind to a Therapeutic protein and that may correspond to a  
5 Therapeutic protein portion of an albumin fusion protein of the invention may also be described or specified in terms of their cross-reactivity. Antibodies that do not bind any other analog, ortholog, or homolog of a Therapeutic protein are included. Antibodies that bind polypeptides with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as  
10 calculated using methods known in the art and described herein) to a Therapeutic protein are also included in the present invention. In specific embodiments, antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention cross-react with murine, rat and/or rabbit homologs of human proteins and the corresponding epitopes thereof. Antibodies that do  
15 not bind polypeptides with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a Therapeutic protein are also included in the present invention. In a specific embodiment, the above-described cross-reactivity is with respect to any single specific antigenic or  
20 immunogenic polypeptide, or combination(s) of 2, 3, 4, 5, or more of the specific antigenic and/or immunogenic polypeptides disclosed herein. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has similar or substantially identical cross reactivity characteristics compared to the .

25 Further included in the present invention are antibodies which bind polypeptides encoded by polynucleotides which hybridize to a polynucleotide encoding a Therapeutic protein under stringent hybridization conditions (as described herein). Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may also be described or specified in terms of  
30 their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M. More preferred binding affinities include those with a dissociation

constant or  $K_d$  less than  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M or  $10^{-8}$  M. Even more preferred binding affinities include those with a dissociation constant or  $K_d$  less than  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M. In

5 preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has an affinity for a given protein or epitope similar to that of the corresponding antibody (not fused to albumin) that binds a Therapeutic protein, taking into account the valency of the albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) and the valency of the corresponding antibody.

The invention also provides antibodies that competitively inhibit binding of an antibody to an epitope of a Therapeutic protein as determined by any method known in the art for determining competitive binding, for example, the immunoassays described herein.

In preferred embodiments, the antibody competitively inhibits binding to the epitope by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, competitively inhibits binding of an antibody to an epitope of a Therapeutic protein as well as the , competitively inhibits binding of an antibody to an epitope of a Therapeutic protein. In other preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, competitively inhibits binding of the to an epitope of a Therapeutic protein by at least 95%, at least 90%, at least 85 %, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50%.

Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may act as agonists or antagonists of the Therapeutic protein. For example, the present invention includes antibodies which disrupt the receptor/ligand interactions with the polypeptides of the invention either partially or fully. The invention features both receptor-specific antibodies and ligand-specific antibodies. The invention also features receptor-specific antibodies which do not prevent ligand binding but prevent receptor activation. Receptor activation (i.e., signaling) may be determined by techniques described herein or otherwise known in the art. For example, receptor activation can be determined by detecting the

phosphorylation (e.g., tyrosine or serine/threonine) of the receptor or its substrate by immunoprecipitation followed by western blot analysis (for example, as described *supra*).

In specific embodiments, antibodies are provided that inhibit ligand activity or receptor activity by at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 60%, or at least 50% of the activity in absence of the antibody. In preferred embodiments, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, has similar or substantially similar characteristics with regard to preventing ligand binding and/or preventing receptor activation compared to the .

The invention also features receptor-specific antibodies which both prevent ligand binding and receptor activation as well as antibodies that recognize the receptor-ligand complex, and, preferably, do not specifically recognize the unbound receptor or the unbound ligand. Likewise, included in the invention are neutralizing antibodies which bind the ligand and prevent binding of the ligand to the receptor, as well as antibodies which bind the ligand, thereby preventing receptor activation, but do not prevent the ligand from binding the receptor. Further included in the invention are antibodies which activate the receptor. These antibodies may act as receptor agonists, i.e., potentiate or activate either all or a subset of the biological activities of the ligand-mediated receptor activation, for example, by inducing dimerization of the receptor. The antibodies may be specified as agonists, antagonists or inverse agonists for biological activities comprising the specific biological activities of the Therapeutic proteins (e.g. as disclosed in Table 1). The above antibody agonists can be made using methods known in the art. See, e.g., PCT publication WO 96/40281; U.S. Patent No. 5,811,097; Deng et al., Blood 92(6):1981-1988 (1998); Chen et al., Cancer Res. 58(16):3668-3678 (1998); Harrop et al., J. Immunol. 161(4):1786-1794 (1998); Zhu et al., Cancer Res. 58(15):3209-3214 (1998); Yoon et al., J. Immunol. 160(7):3170-3179 (1998); Prat et al., J. Cell. Sci. 111(Pt2):237-247 (1998); Pitard et al., J. Immunol. Methods 205(2):177-190 (1997); Liautard et al., Cytokine 9(4):233-241 (1997); Carlson et al., J. Biol. Chem. 272(17):11295-11301 (1997); Taryman et al., Neuron 14(4):755-762 (1995); Muller et al., Structure 6(9):1153-1167 (1998); Bartunek et al., Cytokine 8(1):14-20 (1996) (which are all incorporated by reference herein in their entireties). In preferred embodiments, albumin fusion proteins comprising

at least a fragment or variant of an antibody that binds a Therapeutic protein, have similar or substantially identical agonist or antagonist properties as the .

Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be used, for example, to purify, detect, and target Therapeutic proteins, including both in *in vitro* and *in vivo* diagnostic and therapeutic methods. For example, the antibodies have utility in immunoassays for qualitatively and quantitatively measuring levels of the Therapeutic protein in biological samples. See, e.g., Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); incorporated by reference herein in its entirety. Likewise, albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, may be used, for example, to purify, detect, and target Therapeutic proteins, including both in *in vitro* and *in vivo* diagnostic and therapeutic methods.

Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein include derivatives that are modified, i.e, by the covalent attachment of any type of molecule to the antibody. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, e.g., by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids. Albumin fusion proteins of the invention may also be modified as described above.

#### *Methods of Producing Antibodies that bind Therapeutic Proteins*

The antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention may be generated by any suitable method known in the art. Polyclonal antibodies to an antigen-of-interest can be produced by various procedures well known in the art. For example, a Therapeutic protein may be administered to various host animals including, but not limited to, rabbits, mice, rats, etc. to induce the production of sera containing polyclonal

antibodies specific for the antigen. Various adjuvants may be used to increase the immunological response, depending on the host species, and include but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art.

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well known in the art. In a non-limiting example, mice can be immunized with a Therapeutic protein or fragment or variant thereof or a cell expressing such a Therapeutic protein or fragment or variant thereof. Once an immune response is detected, e.g., antibodies specific for the antigen are detected in the mouse serum, the mouse spleen is harvested and splenocytes isolated. The splenocytes are then fused by well known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the ATCC. Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding a polypeptide of the invention. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

Accordingly, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma

cell secreting an antibody wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention.

5           Another well known method for producing both polyclonal and monoclonal human B cell lines is transformation using Epstein Barr Virus (EBV). Protocols for generating EBV-transformed B cell lines are commonly known in the art, such as, for example, the protocol outlined in Chapter 7.22 of Current Protocols in Immunology, Coligan et al., Eds., 1994, John Wiley & Sons, NY, which is hereby incorporated in its entirety by  
10 reference. The source of B cells for transformation is commonly human peripheral blood, but B cells for transformation may also be derived from other sources including, but not limited to, lymph nodes, tonsil, spleen, tumor tissue, and infected tissues. Tissues are generally made into single cell suspensions prior to EBV transformation. Additionally, steps may be taken to either physically remove or inactivate T cells (e.g., by treatment with  
15 cyclosporin A) in B cell-containing samples, because T cells from individuals seropositive for anti-EBV antibodies can suppress B cell immortalization by EBV.

          In general, the sample containing human B cells is inoculated with EBV, and cultured for 3-4 weeks. A typical source of EBV is the culture supernatant of the B95-8 cell line (ATCC #VR-1492). Physical signs of EBV transformation can generally be seen  
20 towards the end of the 3-4 week culture period. By phase-contrast microscopy, transformed cells may appear large, clear, hairy and tend to aggregate in tight clusters of cells. Initially, EBV lines are generally polyclonal. However, over prolonged periods of cell cultures, EBV lines may become monoclonal or polyclonal as a result of the selective outgrowth of particular B cell clones. Alternatively, polyclonal EBV transformed lines  
25 may be subcloned (e.g., by limiting dilution culture) or fused with a suitable fusion partner and plated at limiting dilution to obtain monoclonal B cell lines. Suitable fusion partners for EBV transformed cell lines include mouse myeloma cell lines (e.g., SP2/0, X63-Ag8.653), heteromyeloma cell lines (human x mouse; e.g., SPAM-8, SBC-H20, and CB-F7), and human cell lines (e.g., GM 1500, SKO-007, RPMI 8226, and KR-4). Thus, the  
30 present invention also provides a method of generating polyclonal or monoclonal human antibodies against polypeptides of the invention or fragments thereof, comprising EBV-transformation of human B cells.

Antibody fragments which recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')<sub>2</sub> fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')<sub>2</sub> fragments). F(ab')<sub>2</sub> fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

For example, antibodies that bind to a Therapeutic protein can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles which carry the polynucleotide sequences encoding them. In a particular embodiment, such phage can be utilized to display antigen binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make antibodies that bind to a Therapeutic protein include those disclosed in Brinkman et al., J. Immunol. Methods 182:41-50 (1995); Ames et al., J. Immunol. Methods 184:177-186 (1995); Kettleborough et al., Eur. J. Immunol. 24:952-958 (1994); Persic et al., Gene 187 9-18 (1997); Burton et al., Advances in Immunology 57:191-280 (1994); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Patent Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108; each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab'

and F(ab')<sub>2</sub> fragments can also be employed using methods known in the art such as those disclosed in PCT publication WO 92/22324; Mullinax et al., BioTechniques 12(6):864-869 (1992); and Sawai et al., AJRI 34:26-34 (1995); and Better et al., Science 240:1041-1043 (1988) (said references incorporated by reference in their entireties).

5        Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Patents 4,946,778 and 5,258,498; Huston et al., Methods in Enzymology 203:46-88 (1991); Shu et al., PNAS 90:7995-7999 (1993); and Skerra et al., Science 240:1038-1040 (1988). For some uses, including *in vivo* use of antibodies in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. See e.g., Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214  
10 (1986); Gillies et al., (1989) J. Immunol. Methods 125:191-202; U.S. Patent Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety. Humanized antibodies are antibody molecules from non-human species antibody that binds the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and a framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions will be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and  
20 sequence comparison to identify unusual framework residues at particular positions. (See, e.g., Queen et al., U.S. Patent No. 5,585,089; Riechmann et al., Nature 332:323 (1988), which are incorporated herein by reference in their entireties.) Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Patent Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, Molecular Immunology 28(4/5):489-498 (1991); Studnicka et al., Protein Engineering  
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7(6):805-814 (1994); Roguska. et al., PNAS 91:969-973 (1994)), and chain shuffling (U.S. Patent No. 5,565,332).

Completely human antibodies are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Patent Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

Human antibodies can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin genes. For example, the human heavy and light chain immunoglobulin gene complexes may be introduced randomly or by homologous recombination into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells in addition to the human heavy and light chain genes. The mouse heavy and light chain immunoglobulin genes may be rendered non-functional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the JH region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a polypeptide of the invention. Monoclonal antibodies directed against the antigen can be obtained from the immunized, transgenic mice using conventional hybridoma technology. The human immunoglobulin transgenes harbored by the transgenic mice rearrange during B cell differentiation, and subsequently undergo class switching and somatic mutation. Thus, using such a technique, it is possible to produce therapeutically useful IgG, IgA, IgM and IgE antibodies. For an overview of this technology for producing human antibodies, see Lonberg and Huszar, Int. Rev. Immunol. 13:65-93 (1995). For a detailed discussion of this technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO

96/33735; European Patent No. 0 598 877; U.S. Patent Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Freemont, CA) and Genpharm (San Jose, CA) can be engaged to provide human antibodies directed against a selected antigen using technology similar to that described above.

Completely human antibodies which recognize a selected epitope can be generated using a technique referred to as "guided selection." In this approach a selected non-human monoclonal antibody, e.g., a mouse antibody, is used to guide the selection of a completely human antibody recognizing the same epitope. (Jespers et al., Bio/technology 12:899-903 (1988)).

#### *Polynucleotides Encoding Antibodies*

The invention further provides polynucleotides comprising a nucleotide sequence encoding an antibody and fragments thereof. The invention also encompasses polynucleotides that hybridize under stringent or alternatively, under lower stringency hybridization conditions, e.g., as defined *supra*, to polynucleotides that encode an antibody, preferably, that specifically binds to a Therapeutic protein, and more preferably, an antibody that binds to a polypeptide having the amino acid sequence of a "therapeutic protein X as disclosed in the "Exemplary Identifier" column of Table 1.

The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (e.g., as described in Kutmeier et al., BioTechniques 17:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

Alternatively, a polynucleotide encoding an antibody may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the immunoglobulin may be chemically synthesized or obtained

from a suitable source (e.g., an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+ RNA, isolated from, any tissue or cells expressing the antibody, such as hybridoma cells selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, e.g., a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art (See Example 60).

Once the nucleotide sequence and corresponding amino acid sequence of the antibody is determined, the nucleotide sequence of the antibody may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, e.g., recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel et al., eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties ), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, e.g., by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, e.g., into human framework regions to humanize a non-human antibody, as described *supra*. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (see, e.g., Chothia et al., J. Mol. Biol. 278: 457-479 (1998) for a listing of human framework regions). Preferably, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds a polypeptide of the invention. Preferably, as discussed *supra*, one or more amino acid substitutions may be made within the framework regions, and, preferably, the amino acid substitutions improve binding of the antibody to its antigen.

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Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., Proc. Natl. Acad. Sci. 81:851-855 (1984); Neuberger et al., Nature 312:604-608 (1984); Takeda et al., Nature 314:452-454 (1985)) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used. As described *supra*, a chimeric antibody is a molecule in which different portions are derived from different animal species, such as those having a variable region derived from a murine mAb and a human immunoglobulin constant region, e.g., humanized antibodies.

Alternatively, techniques described for the production of single chain antibodies (U.S. Patent No. 4,946,778; Bird, Science 242:423- 42 (1988); Huston et al., Proc. Natl. Acad. Sci. USA 85:5879-5883 (1988); and Ward et al., Nature 334:544-54 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain polypeptide. Techniques for the assembly of functional Fv fragments in *E. coli* may also be used (Skerra et al., Science 242:1038- 1041 (1988)).

#### *Recombinant Expression of Antibodies*

Recombinant expression of an antibody, or fragment, derivative or analog thereof, (e.g., a heavy or light chain of an antibody or a single chain antibody), requires construction of an expression vector containing a polynucleotide that encodes the antibody. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression

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vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, in vitro recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (see, e.g., PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Patent No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, or a single chain antibody, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

A variety of host-expression vector systems may be utilized to express the antibody molecules of the invention. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (e.g., *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (e.g., *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing antibody coding sequences; or mammalian cell systems (e.g., COS,

CHO, BHK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (e.g., metallothionein promoter) or from mammalian viruses (e.g., the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking et al., *Gene* 45:101 (1986); Cockett et al., *Bio/Technology* 8:2 (1990)).

In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther et al., *EMBO J.* 2:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lac Z coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res.* 13:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem.* 24:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the antibody coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, e.g., the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (e.g., region E1 or E3) will result in a recombinant virus that is viable and capable of expressing the antibody molecule in infected hosts. (e.g., see Logan & Shenk, Proc. Natl. Acad. Sci. USA 81:355-359 (1984)). Specific initiation signals may also be required for efficient translation of inserted antibody coding sequences. These signals include the ATG initiation codon and adjacent sequences. Furthermore, the initiation codon must be in phase with the reading frame of the desired coding sequence to ensure translation of the entire insert. These exogenous translational control signals and initiation codons can be of a variety of origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of appropriate transcription enhancer elements, transcription terminators, etc. (see Bittner et al., Methods in Enzymol. 153:51-544 (1987)).

In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (e.g., glycosylation) and processing (e.g., cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed. To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used. Such mammalian host cells include but are not limited to CHO, VERY, BHK, Hela, COS, MDCK, 293, 3T3, WI38, and in particular, breast cancer cell lines such as, for example, BT483, Hs578T, HTB2, BT20 and T47D, and normal mammary gland cell line such as, for example, CRL7030 and Hs578Bst.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of

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replication, host cells can be transformed with DNA controlled by appropriate expression control elements (e.g., promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which express the antibody molecule. Such engineered cell lines may be particularly useful in screening and evaluation of compounds that interact directly or indirectly with the antibody molecule.

A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler et al., Cell 11:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, Proc. Natl. Acad. Sci. USA 48:202 (1992)), and adenine phosphoribosyltransferase (Lowy et al., Cell 22:817 (1980)) genes can be employed in tk-, hgp<sup>r</sup>t- or ap<sup>r</sup>t- cells, respectively. Also, antimetabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler et al., Natl. Acad. Sci. USA 77:357 (1980); O'Hare et al., Proc. Natl. Acad. Sci. USA 78:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, Proc. Natl. Acad. Sci. USA 78:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 Clinical Pharmacy 12:488-505; Wu and Wu, Biotherapy 3:87-95 (1991); Tolstoshev, Ann. Rev. Pharmacol. Toxicol. 32:573-596 (1993); Mulligan, Science 260:926-932 (1993); and Morgan and Anderson, Ann. Rev. Biochem. 62:191-217 (1993); May, 1993, TIB TECH 11(5):155-215 (1993)); and hyg<sup>r</sup>, which confers resistance to hygromycin (Santerre et al., Gene 30:147 (1984)). Methods commonly known in the art of recombinant DNA technology may be routinely applied to select the desired recombinant clone, and such methods are described, for example, in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley & Sons, NY (1993); Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli et al. (eds), Current Protocols in Human Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin et al., J. Mol. Biol. 150:1 (1981), which are incorporated by reference herein in their entireties.



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The expression levels of an antibody molecule can be increased by vector amplification (for a review, see Bebbington and Hentschel, The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning, Vol.3. (Academic Press, New York, 1987)). When a marker in the vector system  
5 expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse et al., Mol. Cell. Biol. 3:257 (1983)).

Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can  
10 be amplified in the presence of the drugs methionine sulfoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NS0) which are glutamine synthase negative. Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g. Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to  
15 prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657 which are incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors that may be used according to the present invention are commercially available from suppliers, including,  
20 for example Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington et al., *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are incorporated in their entireties by reference herein.

The host cell may be co-transfected with two expression vectors of the invention,  
25 the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes, and is capable of expressing, both heavy and light chain polypeptides. In such situations, the light chain should be  
30 placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, Nature 322:52 (1986); Kohler, Proc. Natl. Acad. Sci. USA 77:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

Once an antibody molecule of the invention has been produced by an animal, chemically synthesized, or recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

#### *Modifications of Antibodies*

Antibodies that bind a Therapeutic protein or fragments or variants can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

The present invention further encompasses antibodies or fragments thereof conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a tumor as part of a clinical testing procedure to, e.g., determine the efficacy of a given treatment regimen. Detection can be facilitated by coupling the antibody to a detectable substance. Examples of detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. The detectable substance may be coupled or conjugated either directly to the antibody (or fragment thereof) or indirectly, through an intermediate (such as, for example, a linker known in the art) using techniques known in the art. See, for example, U.S. Patent

No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; 5 examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include <sup>125</sup>I, <sup>131</sup>I, <sup>111</sup>In or <sup>99</sup>Tc. Other examples of 10 detectable substances have been described elsewhere herein.

Further, an antibody of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, <sup>213</sup>Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include 15 paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not 20 limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) 25 and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical 30 chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a

protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574. (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Antibodies may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Techniques for conjugating such therapeutic moiety to antibodies are well known.

See, for example, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), pp. 303-16 (Academic Press 1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* 62:119-58 (1982).

Alternatively, an antibody can be conjugated to a second antibody to form an antibody heteroconjugate as described by Segal in U.S. Patent No. 4,676,980, which is incorporated herein by reference in its entirety.

An antibody, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

#### *Antibody-albumin fusion*

Antibodies that bind to a Therapeutic protein and that may correspond to a Therapeutic protein portion of an albumin fusion protein of the invention include, but are not limited to, antibodies that bind a Therapeutic protein disclosed in the "Therapeutic Protein X" column of Table 1, or a fragment or variant thereof.

5 In specific embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH domain. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic  
10 protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two or three VH CDRs. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR1. In other embodiments, the fragment or variant of an antibody that  
15 immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH CDR2. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VH  
20 CDR3.

In specific embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL domain. In other embodiments, the fragment or variant of an antibody that  
25 immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two or three VL CDRs. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL  
30 CDR1. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL

CDR2. In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, the VL CDR3.

5 In other embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, one, two, three, four, five, or six VH and/or VL CDRs.

10 In preferred embodiments, the fragment or variant of an antibody that immunospecifically binds a Therapeutic protein and that corresponds to a Therapeutic protein portion of an albumin fusion protein comprises, or alternatively consists of, an scFv comprising the VH domain of the Therapeutic antibody, linked to the VL domain of the therapeutic antibody by a peptide linker such as (Gly<sub>4</sub>Ser)<sub>3</sub> (SEQ ID NO:36).

#### 15 *Immunophenotyping*

The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may be utilized for immunophenotyping of cell lines and biological samples. Therapeutic proteins of the present invention may be useful as cell-specific markers, or more specifically as cellular markers that are differentially expressed at various stages of differentiation and/or maturation of particular cell types. Monoclonal antibodies (or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) directed against a specific epitope, or combination of epitopes, will allow for the screening of cellular populations expressing the marker. Various techniques can be utilized using monoclonal antibodies (or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) to screen for cellular populations expressing the marker(s), and include magnetic separation using antibody-coated magnetic beads, "panning" with antibody attached to a solid matrix (i.e., plate), and flow cytometry (See, e.g., U.S. Patent 25 5,985,660; and Morrison *et al.*, *Cell*, 96:737-49 (1999)).

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These techniques allow for the screening of particular populations of cells, such as might be found with hematological malignancies (i.e. minimal residual disease (MRD) in

acute leukemic patients) and "non-self" cells in transplantations to prevent Graft-versus-Host Disease (GVHD). Alternatively, these techniques allow for the screening of hematopoietic stem and progenitor cells capable of undergoing proliferation and/or differentiation, as might be found in human umbilical cord blood.

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*Characterizing Antibodies that bind a Therapeutic Protein and Albumin Fusion Proteins Comprising a Fragment or Variant of an Antibody that binds a Therapeutic Protein*

10 The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may be characterized in a variety of ways. In particular, Albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for the ability to specifically  
15 bind to the same antigens specifically bound by the antibody that binds a Therapeutic protein corresponding to the antibody that binds a Therapeutic protein portion of the albumin fusion protein using techniques described herein or routinely modifying techniques known in the art.

Assays for the ability of the antibodies of the invention or albumin fusion proteins  
20 of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) to (specifically) bind a specific protein or epitope may be performed in solution (e.g., Houghten, Bio/Techniques 13:412-421(1992)), on beads (e.g., Lam, Nature 354:82-84 (1991)), on chips (e.g., Fodor, Nature 364:555-556 (1993)), on bacteria (e.g., U.S. Patent No. 5,223,409), on spores (e.g.,  
25 Patent Nos. 5,571,698; 5,403,484; and 5,223,409), on plasmids (e.g., Cull et al., Proc. Natl. Acad. Sci. USA 89:1865-1869 (1992)) or on phage (e.g., Scott and Smith, Science 249:386-390 (1990); Devlin, Science 249:404-406 (1990); Cwirla et al., Proc. Natl. Acad. Sci. USA 87:6378-6382 (1990); and Felici, J. Mol. Biol. 222:301-310 (1991)) (each of these references is incorporated herein in its entirety by reference). The antibodies of the  
30 invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) may also be assayed for their specificity and affinity for a specific protein or epitope using

or routinely modifying techniques described herein or otherwise known in the art.

The albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be assayed for cross-reactivity with other antigens (e.g., molecules that have sequence/structure conservation with the molecule(s) specifically bound by the antibody that binds a Therapeutic protein (or fragment or variant thereof) corresponding to the Therapeutic protein portion of the albumin fusion protein of the invention) by any method known in the art.

Immunoassays which can be used to analyze (immunospecific) binding and cross-reactivity include, but are not limited to, competitive and non-competitive assay systems using techniques such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, precipitin reactions, gel diffusion precipitin reactions, immunodiffusion assays, agglutination assays, complement-fixation assays, immunoradiometric assays, fluorescent immunoassays, and protein A immunoassays, to name but a few. Such assays are routine and well known in the art (see, e.g., Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York, which is incorporated by reference herein in its entirety). Exemplary immunoassays are described briefly below (but are not intended by way of limitation).

Immunoprecipitation protocols generally comprise lysing a population of cells in a lysis buffer such as RIPA buffer (1% NP-40 or Triton X-100, 1% sodium deoxycholate, 0.1% SDS, 0.15 M NaCl, 0.01 M sodium phosphate at pH 7.2, 1% Trasylol) supplemented with protein phosphatase and/or protease inhibitors (e.g., EDTA, PMSF, aprotinin, sodium vanadate), adding an antibody of the invention or albumin fusion protein of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein (or fragment or variant thereof) to the cell lysate, incubating for a period of time (e.g., 1 to 4 hours) at 40 degrees C, adding protein A and/or protein G sepharose beads (or beads coated with an appropriate anti-idiotypic antibody or anti-albumin antibody in the case when an albumin fusion protein comprising at least a fragment or variant of a Therapeutic antibody) to the cell lysate, incubating for about an hour or more at 40 degrees C, washing the beads in lysis buffer and resuspending the beads in SDS/sample buffer. The ability of the antibody or albumin fusion protein of the invention to immunoprecipitate a particular antigen can be assessed by, e.g., western blot analysis. One of skill in the art would be



knowledgeable as to the parameters that can be modified to increase the binding of the antibody or albumin fusion protein to an antigen and decrease the background (*e.g.*, pre-clearing the cell lysate with sepharose beads). For further discussion regarding immunoprecipitation protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.16.1.

Western blot analysis generally comprises preparing protein samples, electrophoresis of the protein samples in a polyacrylamide gel (*e.g.*, 8%- 20% SDS-PAGE depending on the molecular weight of the antigen), transferring the protein sample from the polyacrylamide gel to a membrane such as nitrocellulose, PVDF or nylon, blocking the membrane in blocking solution (*e.g.*, PBS with 3% BSA or non-fat milk), washing the membrane in washing buffer (*e.g.*, PBS-Tween 20), applying the antibody or albumin fusion protein of the invention (diluted in blocking buffer) to the membrane, washing the membrane in washing buffer, applying a secondary antibody (which recognizes the albumin fusion protein, *e.g.*, an anti-human serum albumin antibody) conjugated to an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) or radioactive molecule (*e.g.*,  $^{32}\text{P}$  or  $^{125}\text{I}$ ) diluted in blocking buffer, washing the membrane in wash buffer, and detecting the presence of the antigen. One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected and to reduce the background noise. For further discussion regarding western blot protocols see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 10.8.1.

ELISAs comprise preparing antigen, coating the well of a 96-well microtiter plate with the antigen, washing away antigen that did not bind the wells, adding the antibody or albumin fusion protein (comprising at least a fragment or variant of an antibody that binds a Therapeutic protein) of the invention conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase) to the wells and incubating for a period of time, washing away unbound or non-specifically bound albumin fusion proteins, and detecting the presence of the antibody or albumin fusion proteins specifically bound to the antigen coating the well. In ELISAs the antibody or albumin fusion protein does not have to be conjugated to a detectable compound; instead, a second antibody (which recognizes the antibody or albumin fusion protein, respectively) conjugated to a detectable compound may be added to the well. Further, instead of coating

the well with the antigen, antibody or the albumin fusion protein may be coated to the well. In this case, the detectable molecule could be the antigen conjugated to a detectable compound such as an enzymatic substrate (*e.g.*, horseradish peroxidase or alkaline phosphatase). One of skill in the art would be knowledgeable as to the parameters that can be modified to increase the signal detected as well as other variations of ELISAs known in the art. For further discussion regarding ELISAs see, *e.g.*, Ausubel et al, eds, 1994, Current Protocols in Molecular Biology, Vol. 1, John Wiley & Sons, Inc., New York at 11.2.1.

The binding affinity of an albumin fusion protein to a protein, antigen, or epitope and the off-rate of an antibody- or albumin fusion protein-protein/antigen/epitope interaction can be determined by competitive binding assays. One example of a competitive binding assay is a radioimmunoassay comprising the incubation of labeled antigen (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) with the antibody or albumin fusion protein of the invention in the presence of increasing amounts of unlabeled antigen, and the detection of the antibody bound to the labeled antigen. The affinity of the antibody or albumin fusion protein of the present invention for a specific protein, antigen, or epitope and the binding off-rates can be determined from the data by Scatchard plot analysis. Competition with a second protein that binds the same protein, antigen or epitope as the antibody or albumin fusion protein, can also be determined using radioimmunoassays. In this case, the protein, antigen or epitope is incubated with an antibody or albumin fusion protein of the present invention conjugated to a labeled compound (*e.g.*,  $^3\text{H}$  or  $^{125}\text{I}$ ) in the presence of increasing amounts of an unlabeled second protein that binds the same protein, antigen, or epitope as the albumin fusion protein of the invention.

In a preferred embodiment, BIAcore kinetic analysis is used to determine the binding on and off rates of antibody or albumin fusion proteins of the invention to a protein, antigen or epitope. BIAcore kinetic analysis comprises analyzing the binding and dissociation of antibodies, albumin fusion proteins, or specific polypeptides, antigens or epitopes from chips with immobilized specific polypeptides, antigens or epitopes, antibodies or albumin fusion proteins, respectively, on their surface.

#### *Therapeutic Uses*

The present invention is further directed to antibody-based therapies which involve administering antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein to an animal, preferably a mammal, and most preferably a human, patient for treating one or more of the disclosed diseases, disorders, or conditions. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (including fragments, analogs and derivatives thereof as described herein), nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein), albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein, and nucleic acids encoding such albumin fusion proteins. The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein can be used to treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a Therapeutic protein, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a Therapeutic protein includes, but is not limited to, alleviating symptoms associated with those diseases, disorders or conditions. antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

In a specific and preferred embodiment, the present invention is directed to antibody-based therapies which involve administering antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein to an animal, preferably a mammal, and most preferably a human, patient for treating one or more diseases, disorders, or conditions, including but not limited to: neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions., and/or as described elsewhere herein. Therapeutic compounds of the invention include, but are not limited to, antibodies of the invention (e.g., antibodies

directed to the full length protein expressed on the cell surface of a mammalian cell; antibodies directed to an epitope of a Therapeutic protein and nucleic acids encoding antibodies of the invention (including fragments, analogs and derivatives thereof and anti-idiotypic antibodies as described herein). The antibodies of the invention can be used to  
5 treat, inhibit or prevent diseases, disorders or conditions associated with aberrant expression and/or activity of a Therapeutic protein, including, but not limited to, any one or more of the diseases, disorders, or conditions described herein. The treatment and/or prevention of diseases, disorders, or conditions associated with aberrant expression and/or activity of a Therapeutic protein includes, but is not limited to, alleviating symptoms  
10 associated with those diseases, disorders or conditions. Antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be provided in pharmaceutically acceptable compositions as known in the art or as described herein.

A summary of the ways in which the antibodies of the invention or albumin fusion  
15 proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be used therapeutically includes binding Therapeutic proteins locally or systemically in the body or by direct cytotoxicity of the antibody, e.g. as mediated by complement (CDC) or by effector cells (ADCC). Some of these approaches are described in more detail below. Armed with the teachings provided herein, one of  
20 ordinary skill in the art will know how to use the antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein for diagnostic, monitoring or therapeutic purposes without undue experimentation.

The antibodies of the invention or albumin fusion proteins of the invention  
25 comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be advantageously utilized in combination with other monoclonal or chimeric antibodies, or with lymphokines or hematopoietic growth factors (such as, e.g., IL-2, IL-3 and IL-7), for example, which serve to increase the number or activity of effector cells which interact with the antibodies.

30 The antibodies of the invention or albumin fusion proteins of the invention comprising at least a fragment or variant of an antibody that binds a Therapeutic protein may be administered alone or in combination with other types of treatments (e.g., radiation

therapy, chemotherapy, hormonal therapy, immunotherapy and anti-tumor agents). Generally, administration of products of a species origin or species reactivity (in the case of antibodies) that is the same species as that of the patient is preferred. Thus, in a preferred embodiment, human antibodies, fragments derivatives, analogs, or nucleic acids, are administered to a human patient for therapy or prophylaxis.

It is preferred to use high affinity and/or potent *in vivo* inhibiting and/or neutralizing antibodies against Therapeutic proteins, fragments or regions thereof, (or the albumin fusion protein correlate of such an antibody) for both immunoassays directed to and therapy of disorders related to polynucleotides or polypeptides, including fragments thereof, of the present invention. Such antibodies, fragments, or regions, will preferably have an affinity for polynucleotides or polypeptides of the invention, including fragments thereof. Preferred binding affinities include dissociation constants or Kd's less than  $5 \times 10^{-2}$  M,  $10^{-2}$  M,  $5 \times 10^{-3}$  M,  $10^{-3}$  M,  $5 \times 10^{-4}$  M,  $10^{-4}$  M. More preferred binding affinities include those with a dissociation constant or Kd less than  $5 \times 10^{-5}$  M,  $10^{-5}$  M,  $5 \times 10^{-6}$  M,  $10^{-6}$  M,  $5 \times 10^{-7}$  M,  $10^{-7}$  M,  $5 \times 10^{-8}$  M or  $10^{-8}$  M. Even more preferred binding affinities include those with a dissociation constant or Kd less than  $5 \times 10^{-9}$  M,  $10^{-9}$  M,  $5 \times 10^{-10}$  M,  $10^{-10}$  M,  $5 \times 10^{-11}$  M,  $10^{-11}$  M,  $5 \times 10^{-12}$  M,  $10^{-12}$  M,  $5 \times 10^{-13}$  M,  $10^{-13}$  M,  $5 \times 10^{-14}$  M,  $10^{-14}$  M,  $5 \times 10^{-15}$  M, or  $10^{-15}$  M.

#### Gene Therapy

In a specific embodiment, nucleic acids comprising sequences encoding antibodies that bind therapeutic proteins or albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein are administered to treat, inhibit or prevent a disease or disorder associated with aberrant expression and/or activity of a Therapeutic protein, by way of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded protein that mediates a therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. Exemplary methods are described in more detail elsewhere in this application.

*Demonstration of Therapeutic or Prophylactic Activity*

The compounds or pharmaceutical compositions of the invention are preferably tested *in vitro*, and then *in vivo* for the desired therapeutic or prophylactic activity, prior to use in humans. For example, *in vitro* assays to demonstrate the therapeutic or prophylactic utility of a compound or pharmaceutical composition include, the effect of a compound on a cell line or a patient tissue sample. The effect of the compound or composition on the cell line and/or tissue sample can be determined utilizing techniques known to those of skill in the art including, but not limited to, rosette formation assays and cell lysis assays. In accordance with the invention, *in vitro* assays which can be used to determine whether administration of a specific compound is indicated, include *in vitro* cell culture assays in which a patient tissue sample is grown in culture, and exposed to or otherwise administered a compound, and the effect of such compound upon the tissue sample is observed.

*Therapeutic/Prophylactic Administration and Composition*

The invention provides methods of treatment, inhibition and prophylaxis by administration to a subject of an effective amount of a compound or pharmaceutical composition of the invention, preferably an antibody. In a preferred embodiment, the compound is substantially purified (e.g., substantially free from substances that limit its effect or produce undesired side-effects). The subject is preferably an animal, including but not limited to animals such as cows, pigs, horses, chickens, cats, dogs, etc., and is preferably a mammal, and most preferably human.

Formulations and methods of administration that can be employed when the compound comprises a nucleic acid or an immunoglobulin are described above; additional appropriate formulations and routes of administration can be selected from among those described herein below.

Various delivery systems are known and can be used to administer a compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the compound, receptor-mediated endocytosis (see, e.g., Wu and Wu, J. Biol. Chem. 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of introduction include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous,

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intranasal, epidural, and oral routes. The compounds or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compounds or compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

In a specific embodiment, it may be desirable to administer the pharmaceutical compounds or compositions of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, e.g., in conjunction with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the invention, care must be taken to use materials to which the protein does not absorb.

In another embodiment, the compound or composition can be delivered in a vesicle, in particular a liposome (see Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 353- 365 (1989); Lopez-Berestein, *ibid.*, pp. 317-327; see generally *ibid.*)

In yet another embodiment, the compound or composition can be delivered in a controlled release system. In one embodiment, a pump may be used (see Langer, *supra*; Sefton, *CRC Crit. Ref. Biomed. Eng.* 14:201 (1987); Buchwald et al., *Surgery* 88:507 (1980); Saudek et al., *N. Engl. J. Med.* 321:574 (1989)). In another embodiment, polymeric materials can be used (see *Medical Applications of Controlled Release*, Langer and Wise (eds.), CRC Pres., Boca Raton, Florida (1974); *Controlled Drug Bioavailability, Drug Product Design and Performance*, Smolen and Ball (eds.), Wiley, New York (1984);

Ranger and Peppas, J., Macromol. Sci. Rev. Macromol. Chem. 23:61 (1983); see also Levy et al., Science 228:190 (1985); During et al., Ann. Neurol. 25:351 (1989); Howard et al., J.Neurosurg. 71:105 (1989)). In yet another embodiment, a controlled release system can be placed in proximity of the therapeutic target, e.g., the brain, thus requiring only a fraction of the systemic dose (see, e.g., Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138 (1984)).

Other controlled release systems are discussed in the review by Langer (Science 249:1527-1533 (1990)).

In a specific embodiment where the compound of the invention is a nucleic acid encoding a protein, the nucleic acid can be administered *in vivo* to promote expression of its encoded protein, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a retroviral vector (see U.S. Patent No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see e.g., Joliot et al., Proc. Natl. Acad. Sci. USA 88:1864-1868 (1991)), etc. Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression, by homologous recombination.

The present invention also provides pharmaceutical compositions. Such compositions comprise a therapeutically effective amount of a compound, and a pharmaceutically acceptable carrier. In a specific embodiment, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica



gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in "Remington's Pharmaceutical Sciences" by E.W. Martin. Such compositions will contain a therapeutically effective amount of the compound, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration.

In a preferred embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

The compounds of the invention can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

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FOI b7D b7E b7F b7G b7H b7I b7J b7K b7L b7M b7N b7O b7P b7Q b7R b7S b7T b7U b7V b7W b7X b7Y b7Z

The amount of the compound of the invention which will be effective in the treatment, inhibition and prevention of a disease or disorder associated with aberrant expression and/or activity of a Therapeutic protein can be determined by standard clinical techniques. In addition, in vitro assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from in vitro or animal model test systems.

For antibodies, the dosage administered to a patient is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight. Preferably, the dosage administered to a patient is between 0.1 mg/kg and 20 mg/kg of the patient's body weight, more preferably 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the invention may be reduced by enhancing uptake and tissue penetration (e.g., into the brain) of the antibodies by modifications such as, for example, lipidation.

#### *Diagnosis and Imaging*

Labeled antibodies and derivatives and analogs thereof that bind a Therapeutic protein (or fragment or variant thereof) (including albumin fusion proteins comprising at least a fragment or variant of an antibody that binds a Therapeutic protein), can be used for diagnostic purposes to detect, diagnose, or monitor diseases, disorders, and/or conditions associated with the aberrant expression and/or activity of Therapeutic protein. The invention provides for the detection of aberrant expression of a Therapeutic protein, comprising (a) assaying the expression of the Therapeutic protein in cells or body fluid of an individual using one or more antibodies specific to the polypeptide interest and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed Therapeutic protein expression level compared to the standard expression level is indicative of aberrant expression.

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The invention provides a diagnostic assay for diagnosing a disorder, comprising (a) assaying the expression of the Therapeutic protein in cells or body fluid of an individual using one or more antibodies specific to the Therapeutic protein or albumin fusion proteins comprising at least a fragment of variant of an antibody specific to a Therapeutic protein, and (b) comparing the level of gene expression with a standard gene expression level, whereby an increase or decrease in the assayed Therapeutic protein gene expression level compared to the standard expression level is indicative of a particular disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Antibodies of the invention or albumin fusion proteins comprising at least a fragment of variant of an antibody specific to a Therapeutic protein can be used to assay protein levels in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen et al., J. Cell. Biol. 105:3087-3096 (1987)). Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine (125I, 121I), carbon (14C), sulfur (35S), tritium (3H), indium (112In), and technetium (99Tc); luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

One facet of the invention is the detection and diagnosis of a disease or disorder associated with aberrant expression of a Therapeutic protein in an animal, preferably a mammal and most preferably a human. In one embodiment, diagnosis comprises: a) administering (for example, parenterally, subcutaneously, or intraperitoneally) to a subject an effective amount of a labeled molecule which specifically binds to the polypeptide of interest; b) waiting for a time interval following the administering for permitting the labeled molecule to preferentially concentrate at sites in the subject where the Therapeutic protein is expressed (and for unbound labeled molecule to be cleared to background level);

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c) determining background level; and d) detecting the labeled molecule in the subject, such that detection of labeled molecule above the background level indicates that the subject has a particular disease or disorder associated with aberrant expression of the therapeutic protein. Background level can be determined by various methods including, comparing the amount of labeled molecule detected to a standard value previously determined for a particular system.

It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99m</sup>Tc. The labeled antibody, antibody fragment, or albumin fusion protein comprising at least a fragement or variant of an antibody that binds a Therapeutic protein will then preferentially accumulate at the location of cells which contain the specific Therapeutic protein. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments." (Chapter 13 in Tumor Imaging: The Radiochemical Detection of Cancer, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

Depending on several variables, including the type of label used and the mode of administration, the time interval following the administration for permitting the labeled molecule to preferentially concentrate at sites in the subject and for unbound labeled molecule to be cleared to background level is 6 to 48 hours or 6 to 24 hours or 6 to 12 hours. In another embodiment the time interval following administration is 5 to 20 days or 5 to 10 days.

In an embodiment, monitoring of the disease or disorder is carried out by repeating the method for diagnosing the disease or disease, for example, one month after initial diagnosis, six months after initial diagnosis, one year after initial diagnosis, etc.

Presence of the labeled molecule can be detected in the patient using methods known in the art for *in vivo* scanning. These methods depend upon the type of label used. Skilled artisans will be able to determine the appropriate method for detecting a particular label. Methods and devices that may be used in the diagnostic methods of the invention include, but are not limited to, computed tomography (CT), whole body scan such as

position emission tomography (PET), magnetic resonance imaging (MRI), and sonography.

In a specific embodiment, the molecule is labeled with a radioisotope and is detected in the patient using a radiation responsive surgical instrument (Thurston et al.,  
5 U.S. Patent No. 5,441,050). In another embodiment, the molecule is labeled with a fluorescent compound and is detected in the patient using a fluorescence responsive scanning instrument. In another embodiment, the molecule is labeled with a positron emitting metal and is detected in the patient using positron emission-tomography. In yet another embodiment, the molecule is labeled with a paramagnetic label and is detected in a  
10 patient using magnetic resonance imaging (MRI).

#### *Kits*

The present invention provides kits that can be used in the above methods. In one embodiment, a kit comprises an antibody, preferably a purified antibody, in one or more  
15 containers. In a specific embodiment, the kits of the present invention contain a substantially isolated polypeptide comprising an epitope which is specifically immunoreactive with an antibody included in the kit. Preferably, the kits of the present invention further comprise a control antibody which does not react with the polypeptide of interest. In another specific embodiment, the kits of the present invention contain a means  
20 for detecting the binding of an antibody to a polypeptide of interest (e.g., the antibody may be conjugated to a detectable substrate such as a fluorescent compound, an enzymatic substrate, a radioactive compound or a luminescent compound, or a second antibody which recognizes the first antibody may be conjugated to a detectable substrate).

In another specific embodiment of the present invention, the kit is a diagnostic kit  
25 for use in screening serum containing antibodies specific against proliferative and/or cancerous polynucleotides and polypeptides. Such a kit may include a control antibody that does not react with the polypeptide of interest. Such a kit may include a substantially isolated polypeptide antigen comprising an epitope which is specifically immunoreactive with at least one anti-polypeptide antigen antibody. Further, such a kit includes means for  
30 detecting the binding of said antibody to the antigen (e.g., the antibody may be conjugated to a fluorescent compound such as fluorescein or rhodamine which can be detected by flow cytometry). In specific embodiments, the kit may include a recombinantly produced

or chemically synthesized polypeptide antigen. The polypeptide antigen of the kit may also be attached to a solid support.

In a more specific embodiment the detecting means of the above-described kit includes a solid support to which said polypeptide antigen is attached. Such a kit may also include a non-attached reporter-labeled anti-human antibody. In this embodiment, binding of the antibody to the polypeptide antigen can be detected by binding of the said reporter-labeled antibody.

In an additional embodiment, the invention includes a diagnostic kit for use in screening serum containing antigens of the polypeptide of the invention. The diagnostic kit includes a substantially isolated antibody specifically immunoreactive with polypeptide or polynucleotide antigens, and means for detecting the binding of the polynucleotide or polypeptide antigen to the antibody. In one embodiment, the antibody is attached to a solid support. In a specific embodiment, the antibody may be a monoclonal antibody. The detecting means of the kit may include a second, labeled monoclonal antibody. Alternatively, or in addition, the detecting means may include a labeled, competing antigen.

In one diagnostic configuration, test serum is reacted with a solid phase reagent having a surface-bound antigen obtained by the methods of the present invention. After binding with specific antigen antibody to the reagent and removing unbound serum components by washing, the reagent is reacted with reporter-labeled anti-human antibody to bind reporter to the reagent in proportion to the amount of bound anti-antigen antibody on the solid support. The reagent is again washed to remove unbound labeled antibody, and the amount of reporter associated with the reagent is determined. Typically, the reporter is an enzyme which is detected by incubating the solid phase in the presence of a suitable fluorometric, luminescent or colorimetric substrate (Sigma, St. Louis, MO).

The solid surface reagent in the above assay is prepared by known techniques for attaching protein material to solid support material, such as polymeric beads, dip sticks, 96-well plate or filter material. These attachment methods generally include non-specific adsorption of the protein to the support or covalent attachment of the protein, typically through a free amine group, to a chemically reactive group on the solid support, such as an activated carboxyl, hydroxyl, or aldehyde group. Alternatively, streptavidin coated plates can be used in conjunction with biotinylated antigen(s).

Thus, the invention provides an assay system or kit for carrying out this diagnostic method. The kit generally includes a support with surface-bound recombinant antigens, and a reporter-labeled anti-human antibody for detecting surface-bound anti-antigen antibody.

5

### **Albumin Fusion Proteins**

The present invention relates generally to albumin fusion proteins and methods of treating, preventing, or ameliorating diseases or disorders. As used herein, "albumin fusion protein" refers to a protein formed by the fusion of at least one molecule of albumin (or a fragment or variant thereof) to at least one molecule of a Therapeutic protein (or fragment or variant thereof). An albumin fusion protein of the invention comprises at least a fragment or variant of a Therapeutic protein and at least a fragment or variant of human serum albumin, which are associated with one another, preferably by genetic fusion (i.e., the albumin fusion protein is generated by translation of a nucleic acid in which a polynucleotide encoding all or a portion of a Therapeutic protein is joined in-frame with a polynucleotide encoding all or a portion of albumin) or chemical conjugation to one another. The Therapeutic protein and albumin protein, once part of the albumin fusion protein, may be referred to as a "portion", "region" or "moiety" of the albumin fusion protein.

In one embodiment, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein (e.g., as described in Table 1) and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment of a Therapeutic protein and a serum albumin protein. In other embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active variant of a Therapeutic protein and a serum albumin protein. In preferred embodiments, the serum albumin protein component of the albumin fusion protein is the mature portion of serum albumin.

In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein, and a biologically active

and/or therapeutically active fragment of serum albumin. In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a Therapeutic protein and a biologically active and/or therapeutically active variant of serum albumin. In preferred embodiments, the Therapeutic protein portion of the albumin fusion protein is the mature portion of the Therapeutic protein.

In further embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, a biologically active and/or therapeutically active fragment or variant of a Therapeutic protein and a biologically active and/or therapeutically active fragment or variant of serum albumin. In preferred embodiments, the invention provides an albumin fusion protein comprising, or alternatively consisting of, the mature portion of a Therapeutic protein and the mature portion of serum albumin.

Preferably, the albumin fusion protein comprises HA as the N-terminal portion, and a Therapeutic protein as the C-terminal portion. Alternatively, an albumin fusion protein comprising HA as the C-terminal portion, and a Therapeutic protein as the N-terminal portion may also be used.

In other embodiments, the albumin fusion protein has a Therapeutic protein fused to both the N-terminus and the C-terminus of albumin. In a preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are the same Therapeutic proteins. In a preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins. In another preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins which may be used to treat or prevent the same disease, disorder, or condition (e.g. as listed in the "Preferred Indication Y" column of Table 1). In another preferred embodiment, the Therapeutic proteins fused at the N- and C- termini are different Therapeutic proteins which may be used to treat or prevent diseases or disorders (e.g. as listed in the "Preferred Indication Y" column of Table 1) which are known in the art to commonly occur in patients simultaneously.

In addition to albumin fusion protein in which the albumin portion is fused N-terminal and/or C-terminal of the Therapeutic protein portion, albumin fusion proteins of the invention may also be produced by inserting the Therapeutic protein or peptide of interest (e.g., a Therapeutic protein X as disclosed in Table 1, or an antibody that binds a Therapeutic protein or a fragment or variant thereof) into an internal region of HA. For



instance, within the protein sequence of the HA molecule a number of loops or turns exist between the end and beginning of  $\alpha$ -helices, which are stabilized by disulphide bonds (see Figures 9-11). The loops, as determined from the crystal structure of HA (Fig. 13) (PDB identifiers 1AO6, 1BJ5, 1BKE, 1BM0, 1E7E to 1E7I and 1UOR) for the most part extend  
5 away from the body of the molecule. These loops are useful for the insertion, or internal fusion, of therapeutically active peptides, particularly those requiring a secondary structure to be functional, or Therapeutic proteins, to essentially generate an albumin molecule with specific biological activity.

Loops in human albumin structure into which peptides or polypeptides may be  
10 inserted to generate albumin fusion proteins of the invention include: Val54-Asn61, Thr76-Asp89, Ala92-Glu100, Gln170-Ala176, His 247 - Glu252, Glu 266 - Glu277, Glu 280-His288, Ala362-Glu368, Lys439-Pro447, Val462-Lys475, Thr478-Pro486, and Lys560-Thr566. In more preferred embodiments, peptides or polypeptides are inserted into the Val54-Asn61, Gln170-Ala176, and/or Lys560-Thr566 loops of mature human  
15 albumin (SEQ ID NO:18).

Peptides to be inserted may be derived from either phage display or synthetic peptide libraries screened for specific biological activity or from the active portions of a molecule with the desired function. Additionally, random peptide libraries may be generated within particular loops or by insertions of randomized peptides into particular  
20 loops of the HA molecule and in which all possible combinations of amino acids are represented.

Such library(s) could be generated on HA or domain fragments of HA by one of the following methods:

(a) randomized mutation of amino acids within one or more peptide loops of  
25 HA or HA domain fragments. Either one, more or all the residues within a loop could be mutated in this manner (for example see Fig. 10a);

(b) replacement of, or insertion into one or more loops of HA or HA domain fragments (*i.e.*, internal fusion) of a randomized peptide(s) of length  $X_n$  (where X is an amino acid and n is the number of residues (for example see Fig. 10b);

30 (c) N-, C- or N- and C- terminal peptide/protein fusions in addition to (a) and/or (b).

The HA or HA domain fragment may also be made multifunctional by grafting the

peptides derived from different screens of different loops against different targets into the same HA or HA domain fragment.

In preferred embodiments, peptides inserted into a loop of human serum albumin are peptide fragments or peptide variants of the Therapeutic proteins disclosed in Table 1.

5 More particularly, the invention encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids in length inserted into a loop of human serum albumin. The invention also encompasses albumin fusion proteins which comprise peptide fragments or  
10 peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids fused to the N-terminus of human serum albumin. The invention also encompasses albumin fusion proteins which comprise peptide fragments or peptide variants at least 7 at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at  
15 least 14, at least 15, at least 20, at least 25, at least 30, at least 35, or at least 40 amino acids fused to the C-terminus of human serum albumin.

Generally, the albumin fusion proteins of the invention may have one HA-derived region and one Therapeutic protein-derived region. Multiple regions of each protein, however, may be used to make an albumin fusion protein of the invention. Similarly,  
20 more than one Therapeutic protein may be used to make an albumin fusion protein of the invention. For instance, a Therapeutic protein may be fused to both the N- and C-terminal ends of the HA. In such a configuration, the Therapeutic protein portions may be the same or different Therapeutic protein molecules. The structure of bifunctional albumin fusion proteins may be represented as: X-HA-Y or Y-HA-X.

25 For example, an anti-BLyS™ scFv-HA-IFN $\alpha$ -2b fusion may be prepared to modulate the immune response to IFN $\alpha$ -2b by anti-BLyS™ scFv. An alternative is making a bi (or even multi) functional dose of HA-fusions e.g. HA-IFN $\alpha$ -2b fusion mixed with HA-anti-BLyS™ scFv fusion or other HA-fusions in various ratio's depending on function, half-life etc.

30 Bi- or multi-functional albumin fusion proteins may also be prepared to target the Therapeutic protein portion of a fusion to a target organ or cell type via protein or peptide at the opposite terminus of HA.

As an alternative to the fusion of known therapeutic molecules, the peptides could be obtained by screening libraries constructed as fusions to the N-, C- or N- and C- termini of HA, or domain fragment of HA, of typically 6, 8, 12, 20 or 25 or  $X_n$  (where X is an amino acid (aa) and n equals the number of residues) randomized amino acids, and in which all possible combinations of amino acids were represented. A particular advantage of this approach is that the peptides may be selected *in situ* on the HA molecule and the properties of the peptide would therefore be as selected for rather than, potentially, modified as might be the case for a peptide derived by any other method then being attached to HA.

Additionally, the albumin fusion proteins of the invention may include a linker peptide between the fused portions to provide greater physical separation between the moieties and thus maximize the accessibility of the Therapeutic protein portion, for instance, for binding to its cognate receptor. The linker peptide may consist of amino acids such that it is flexible or more rigid.

The linker sequence may be cleavable by a protease or chemically to yield the growth hormone related moiety. Preferably, the protease is one which is produced naturally by the host, for example the *S. cerevisiae* protease *kex2* or equivalent proteases.

Therefore, as described above, the albumin fusion proteins of the invention may have the following formula R1-L-R2; R2-L-R1; or R1-L-R2-L-R1, wherein R1 is at least one Therapeutic protein, peptide or polypeptide sequence, and not necessarily the same Therapeutic protein, L is a linker and R2 is a serum albumin sequence.

In preferred embodiments, Albumin fusion proteins of the invention comprising a Therapeutic protein have extended shelf life compared to the shelf life the same Therapeutic protein when not fused to albumin. Shelf-life typically refers to the time period over which the therapeutic activity of a Therapeutic protein in solution or in some other storage formulation, is stable without undue loss of therapeutic activity. Many of the Therapeutic proteins are highly labile in their unfused state. As described below, the typical shelf-life of these Therapeutic proteins is markedly prolonged upon incorporation into the albumin fusion protein of the invention.

Albumin fusion proteins of the invention with "prolonged" or "extended" shelf-life exhibit greater therapeutic activity relative to a standard that has been subjected to the same storage and handling conditions. The standard may be the unfused full-length

Therapeutic protein. When the Therapeutic protein portion of the albumin fusion protein is an analog, a variant, or is otherwise altered or does not include the complete sequence for that protein, the prolongation of therapeutic activity may alternatively be compared to the unfused equivalent of that analog, variant, altered peptide or incomplete sequence. As an example, an albumin fusion protein of the invention may retain greater than about 100% of the therapeutic activity, or greater than about 105%, 110%, 120%, 130%, 150% or 200% of the therapeutic activity of a standard when subjected to the same storage and handling conditions as the standard when compared at a given time point.

Shelf-life may also be assessed in terms of therapeutic activity remaining after storage, normalized to therapeutic activity when storage began. Albumin fusion proteins of the invention with prolonged or extended shelf-life as exhibited by prolonged or extended therapeutic activity may retain greater than about 50% of the therapeutic activity, about 60%, 70%, 80%, or 90% or more of the therapeutic activity of the equivalent unfused Therapeutic protein when subjected to the same conditions. For example, as discussed in Example 1, an albumin fusion protein of the invention comprising hGH fused to the full length HA sequence may retain about 80% or more of its original activity in solution for periods of up to 5 weeks or more under various temperature conditions.

#### ***Expression of Fusion Proteins***

The albumin fusion proteins of the invention may be produced as recombinant molecules by secretion from yeast, a microorganism such as a bacterium, or a human or animal cell line. Preferably, the polypeptide is secreted from the host cells. We have found that, by fusing the hGH coding sequence to the HA coding sequence, either to the 5' end or 3' end, it is possible to secrete the albumin fusion protein from yeast without the requirement for a yeast-derived pro sequence. This was surprising, as other workers have found that a yeast derived pro sequence was needed for efficient secretion of hGH in yeast.

For example, Hiramatsu *et al.* (Appl Environ Microbiol 56:2125 (1990); Appl Environ Microbiol 57:2052 (1991)) found that the N-terminal portion of the pro sequence in the *Mucor pusillus* rennin pre-pro leader was important. Other authors, using the MF -1 signal, have always included the MF -1 pro sequence when secreting hGH. The pro sequences were believed to assist in the folding of the hGH by acting as an intramolecular

chaperone. The present invention shows that HA or fragments of HA can perform a similar function.

Hence, a particular embodiment of the invention comprises a DNA construct encoding a signal sequence effective for directing secretion in yeast, particularly a yeast-derived signal sequence (especially one which is homologous to the yeast host), and the fused molecule of the first aspect of the invention, there being no yeast-derived pro sequence between the signal and the mature polypeptide.

The *Saccharomyces cerevisiae* invertase signal is a preferred example of a yeast-derived signal sequence.

Conjugates of the kind prepared by Poznansky *et al.*, (FEBS Lett. 239:18 (1988)), in which separately-prepared polypeptides are joined by chemical cross-linking, are not contemplated.

The present invention also includes a cell, preferably a yeast cell transformed to express an albumin fusion protein of the invention. In addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. If the polypeptide is secreted, the medium will contain the polypeptide, with the cells, or without the cells if they have been filtered or centrifuged away. Many expression systems are known and may be used, including bacteria (for example *E. coli* and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Pichia pastoris*, filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

Preferred yeast strains to be used in the production of albumin fusion proteins are D88, DXY1 and BXP10. D88 [*leu2-3, leu2-122, can1, pral, ubc4*] is a derivative of parent strain AH22<sup>his+</sup> (also known as DB1; see, e.g., Sleep *et al.* Biotechnology 8:42-46 (1990)). The strain contains a *leu2* mutation which allows for auxotrophic selection of 2 micron-based plasmids that contain the LEU2 gene. D88 also exhibits a derepression of PRB1 in glucose excess. The PRB1 promoter is normally controlled by two checkpoints that monitor glucose levels and growth stage. The promoter is activated in wild type yeast upon glucose depletion and entry into stationary phase. Strain D88 exhibits the repression by glucose but maintains the induction upon entry into stationary phase. The PRA1 gene encodes a yeast vacuolar protease, YscA endoprotease A, that is localized in the ER. The

UBC4 gene is in the ubiquitination pathway and is involved in targeting short lived and abnormal proteins for ubiquitin dependant degradation. Isolation of this *ubc4* mutation was found to increase the copy number of an expression plasmid in the cell and cause an increased level of expression of a desired protein expressed from the plasmid (see, e.g., International Publication No. WO99/00504, hereby incorporated in its entirety by reference herein).

DXY1, a derivative of D88, has the following genotype: [*leu2-3*, *leu2-122*, *can1*, *pral*, *ubc4*, *ura3::yap3*]. In addition to the mutations isolated in D88, this strain also has a knockout of the YAP3 protease. This protease causes cleavage of mostly di-basic residues (RR, RK, KR, KK) but can also promote cleavage at single basic residues in proteins. Isolation of this *yap3* mutation resulted in higher levels of full length HSA production (see, e.g., U.S. Patent No. 5,965,386 and Kerry-Williams et al., Yeast 14:161-169 (1998), hereby incorporated in their entireties by reference herein).

BXP10 has the following genotype: *leu2-3*, *leu2-122*, *can1*, *pral*, *ubc4*, *ura3*, *yap3::URA3*, *lys2*, *hsp150::LYS2*, *pmt1::URA3*. In addition to the mutations isolated in DXY1, this strain also has a knockout of the PMT1 gene and the HSP150 gene. The PMT1 gene is a member of the evolutionarily conserved family of dolichyl-phosphate-D-mannose protein O-mannosyltransferases (Pmts). The transmembrane topology of Pmt1p suggests that it is an integral membrane protein of the endoplasmic reticulum with a role in O-linked glycosylation. This mutation serves to reduce/eliminate O-linked glycosylation of HSA fusions (see, e.g., International Publication No. WO00/44772, hereby incorporated in its entirety by reference herein). Studies revealed that the Hsp150 protein is inefficiently separated from rHA by ion exchange chromatography. The mutation in the HSP150 gene removes a potential contaminant that has proven difficult to remove by standard purification techniques. See, e.g., U.S. Patent No. 5,783,423, hereby incorporated in its entirety by reference herein.

The desired protein is produced in conventional ways, for example from a coding sequence inserted in the host chromosome or on a free plasmid. The yeasts are transformed with a coding sequence for the desired protein in any of the usual ways, for example electroporation. Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) *Methods Enzymol.* 194, 182.

Successfully transformed cells, *i.e.*, cells that contain a DNA construct of the

present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an expression construct can be grown to produce the desired polypeptide. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* 98, 503 or Berent *et al.* (1985) *Biotech.* 3, 208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

Useful yeast plasmid vectors include pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers HIS3, 7RP1, LEU2 and URA3. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

Preferred vectors for making albumin fusion proteins for expression in yeast include pPPC0005, pScCHSA, pScNHSA, and pC4:HSA which are described in detail in Example 2. Figure 4 shows a map of the pPPC0005 plasmid that can be used as the base vector into which polynucleotides encoding Therapeutic proteins may be cloned to form HA-fusions. It contains a *PRB1 S. cerevisiae* promoter (PRB1p), a Fusion leader sequence (FL), DNA encoding HA (rHA) and an *ADH1 S. cerevisiae* terminator sequence. The sequence of the fusion leader sequence consists of the first 19 amino acids of the signal peptide of human serum albumin (SEQ ID NO:29) and the last five amino acids of the mating factor alpha 1 promoter (SLDKR, see EP-A-387 319 which is hereby incorporated by reference in its entirety).

The plasmids, pPPC0005, pScCHSA, pScNHSA, and pC4:HSA were deposited on April 11, 2001 at the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 and given accession numbers ATCC \_\_\_\_\_, \_\_\_\_\_, \_\_\_\_\_, and \_\_\_\_\_, respectively. Another vector useful for expressing an albumin fusion protein in yeast the pSAC35 vector which is described in Sleep *et al.*, *BioTechnology* 8:42 (1990) which is hereby incorporated by reference in its entirety.

A variety of methods have been developed to operably link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

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Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion, is treated with bacteriophage T4 DNA polymerase or E. coli DNA polymerase I, enzymes that remove protruding, -single-stranded termini with their 3' 5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

15 Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc, New Haven, CT, USA.

20 A desirable way to modify the DNA in accordance with the invention, if, for example, HA variants are to be prepared, is to use the polymerase chain reaction as disclosed by Saiki *et al.* (1988) *Science* 239, 487-491. In this method the DNA to be enzymatically amplified is flanked by two specific oligonucleotide primers which themselves become incorporated into the amplified DNA. The specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

25 Exemplary genera of yeast contemplated to be useful in the practice of the present invention as hosts for expressing the albumin fusion proteins are *Pichia* (Hansenula), *Saccharomyces*, *Kluyveromyces*, *Candida*, *Torulopsis*, *Torulaspora*, *Schizosaccharomyces*, *Citeromyces*, *Pachysolen*, *Debaromyces*, *Metschnikowia*, *Rhodospiridium*, *Leucosporidium*, *Botryoascus*, *Sporidiobolus*, *Endomycopsis*, and the  
30 like. Preferred genera are those selected from the group consisting of *Saccharomyces*, *Schizosaccharomyces*, *Kluyveromyces*, *Pichia* and *Torulaspora*. Examples of *Saccharomyces* spp. are *S. cerevisiae*, *S. italicus* and *S. rouxii*.



Examples of *Kluyveromyces* spp. are *K. fragilis*, *K. lactis* and *K. marxianus*. A suitable *Torulaspora* species is *T. delbrueckii*. Examples of *Pichia* (Hansenula) spp. are *P. angusta* (formerly *H. polymorpha*), *P. anomala* (formerly *H. anomala*) and *P. pastoris*. Methods for the transformation of *S. cerevisiae* are taught generally in EP 251 744, EP 5 258 067 and WO 90/01063, all of which are incorporated herein by reference.

Preferred exemplary species of *Saccharomyces* include *S. cerevisiae*, *S. italicus*, *S. diastaticus*, and *Zygosaccharomyces rouxii*. Preferred exemplary species of *Kluyveromyces* include *K. fragilis* and *K. lactis*. Preferred exemplary species of *Hansenula* include *H. polymorpha* (now *Pichia angusta*), *H. anomala* (now *Pichia* 10 *anomala*), and *Pichia capsulata*. Additional preferred exemplary species of *Pichia* include *P. pastoris*. Preferred exemplary species of *Aspergillus* include *A. niger* and *A. nidulans*. Preferred exemplary species of *Yarrowia* include *Y. lipolytica*. Many preferred yeast species are available from the ATCC. For example, the following preferred yeast species are available from the ATCC and are useful in the expression of albumin fusion 15 proteins: *Saccharomyces cerevisiae* Hansen, teleomorph strain BY4743 *yap3* mutant (ATCC Accession No. 4022731); *Saccharomyces cerevisiae* Hansen, teleomorph strain BY4743 *hsp150* mutant (ATCC Accession No. 4021266); *Saccharomyces cerevisiae* Hansen, teleomorph strain BY4743 *pmt1* mutant (ATCC Accession No. 4023792); *Saccharomyces cerevisiae* Hansen, teleomorph (ATCC Accession Nos. 20626; 44773; 20 44774; and 62995); *Saccharomyces diastaticus* Andrews et Gilliland ex van der Walt, teleomorph (ATCC Accession No. 62987); *Kluyveromyces lactis* (Dombrowski) van der Walt, teleomorph (ATCC Accession No. 76492); *Pichia angusta* (Teunisson et al.) Kurtzman, teleomorph deposited as *Hansenula polymorpha* de Morais et Maia, teleomorph (ATCC Accession No. 26012); *Aspergillus niger* van Tieghem, anamorph 25 (ATCC Accession No. 9029); *Aspergillus niger* van Tieghem, anamorph (ATCC Accession No. 16404); *Aspergillus nidulans* (Eidam) Winter, anamorph (ATCC Accession No. 48756); and *Yarrowia lipolytica* (Wickerham et al.) van der Walt et von Arx, teleomorph (ATCC Accession No. 201847).

Suitable promoters for *S. cerevisiae* include those associated with the PGKI gene, 30 GAL1 or GAL10 genes, CYC1, PHO5, TRP1, ADHI, ADH2, the genes for glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, triose phosphate isomerase, phosphoglucose isomerase, glucokinase,

alpha-mating factor pheromone, [a mating factor pheromone], the PRBI promoter, the GUT2 promoter, the GPDI promoter, and hybrid promoters involving hybrids of parts of 5' regulatory regions with parts of 5' regulatory regions of other promoters or with upstream activation sites (e.g. the promoter of EP-A-258 067).

- 5            Convenient regulatable promoters for use in *Schizosaccharomyces pombe* are the thiamine-repressible promoter from the nmt gene as described by Maundrell (1990) *J. Biol. Chem.* 265, 10857-10864 and the glucose repressible jbp1 gene promoter as described by Hoffman & Winston (1990) *Genetics* 124, 807-816.

- 10           Methods of transforming *Pichia* for expression of foreign genes are taught in, for example, Cregg *et al.* (1993), and various Phillips patents (e.g. US 4 857 467, incorporated herein by reference), and *Pichia* expression kits are commercially available from Invitrogen BV, Leek, Netherlands, and Invitrogen Corp., San Diego, California. Suitable promoters include AOX1 and AOX2. Gleeson *et al.* (1986) *J. Gen. Microbiol.* 132, 3459-3465 include information on *Hansenula* vectors and transformation, suitable  
15 promoters being MOX1 and FMD1; whilst EP 361 991, Flier *et al.* (1991) and other publications from Rhone-Poulenc Rorer teach how to express foreign proteins in *Kluyveromyces* spp., a suitable promoter being PGKI.

- 20           The transcription termination signal is preferably the 3' flanking sequence of a eukaryotic gene which contains proper signals for transcription termination and polyadenylation. Suitable 3' flanking sequences may, for example, be those of the gene naturally linked to the expression control sequence used, *i.e.* may correspond to the promoter. Alternatively, they may be different in which case the termination signal of the *S. cerevisiae* ADHI gene is preferred.

- 25           The desired albumin fusion protein may be initially expressed with a secretion leader sequence, which may be any leader effective in the yeast chosen. Leaders useful in *S. cerevisiae* include that from the mating factor polypeptide (MF -1) and the hybrid leaders of EP-A-387 319. Such leaders (or signals) are cleaved by the yeast before the mature albumin is released into the surrounding medium. Further such leaders include those of *S. cerevisiae* invertase (SUC2) disclosed in JP 62-096086 (granted as  
30 911036516), acid phosphatase (PH05), the pre-sequence of MFoz-1, 0 glucanase (BGL2) and killer toxin; *S. diastaticus* glucoamylase II; *S. carlsbergensis* -galactosidase (MEL1); *K. lactis* killer toxin; and *Candida glucoamylase*.

## Additional Methods of Recombinant and Synthetic Production of Albumin Fusion Proteins

5 The present invention also relates to vectors containing a polynucleotide encoding an albumin fusion protein of the present invention, host cells, and the production of albumin fusion proteins by synthetic and recombinant techniques. The vector may be, for example, a phage, plasmid, viral, or retroviral vector. Retroviral vectors may be replication competent or replication defective. In the latter case, viral propagation generally will occur only in complementing host cells.

10 The polynucleotides encoding albumin fusion proteins of the invention may be joined to a vector containing a selectable marker for propagation in a host. Generally, a plasmid vector is introduced in a precipitate, such as a calcium phosphate precipitate, or in a complex with a charged lipid. If the vector is a virus, it may be packaged in vitro using an appropriate packaging cell line and then transduced into host cells.

15 The polynucleotide insert should be operatively linked to an appropriate promoter, such as the phage lambda PL promoter, the *E. coli lac*, *trp*, *phoA* and *tac* promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters will be known to the skilled artisan. The expression constructs will further contain sites for transcription initiation, termination, and, in the transcribed region,  
20 a ribosome binding site for translation. The coding portion of the transcripts expressed by the constructs will preferably include a translation initiating codon at the beginning and a termination codon (UAA, UGA or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors will preferably include at least one selectable  
25 marker. Such markers include dihydrofolate reductase, G418, glutamine synthase, or neomycin resistance for eukaryotic cell culture, and tetracycline, kanamycin or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces* and *Salmonella typhimurium* cells; fungal cells, such as yeast cells (e.g.,  
30 *Saccharomyces cerevisiae* or *Pichia pastoris* (ATCC Accession No. 201178)); insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS, NSO, 293, and Bowes melanoma cells; and plant cells. Appropriate culture mediums and

conditions for the above-described host cells are known in the art.

Among vectors preferred for use in bacteria include pQE70, pQE60 and pQE-9, available from QIAGEN, Inc.; pBluescript vectors, Phagescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene Cloning Systems, Inc.; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5 available from Pharmacia Biotech, Inc. Among preferred eukaryotic vectors are pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Preferred expression vectors for use in yeast systems include, but are not limited to pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalph, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, pPIC9K, and PAO815 (all available from Invitrogen, Carlsbad, CA). Other suitable vectors will be readily apparent to the skilled artisan.

In one embodiment, polynucleotides encoding an albumin fusion protein of the invention may be fused to signal sequences which will direct the localization of a protein of the invention to particular compartments of a prokaryotic or eukaryotic cell and/or direct the secretion of a protein of the invention from a prokaryotic or eukaryotic cell. For example, in *E. coli*, one may wish to direct the expression of the protein to the periplasmic space. Examples of signal sequences or proteins (or fragments thereof) to which the albumin fusion proteins of the invention may be fused in order to direct the expression of the polypeptide to the periplasmic space of bacteria include, but are not limited to, the *pelB* signal sequence, the maltose binding protein (MBP) signal sequence, MBP, the *ompA* signal sequence, the signal sequence of the periplasmic *E. coli* heat-labile enterotoxin B-subunit, and the signal sequence of alkaline phosphatase. Several vectors are commercially available for the construction of fusion proteins which will direct the localization of a protein, such as the pMAL series of vectors (particularly the pMAL-p series) available from New England Biolabs. In a specific embodiment, polynucleotides albumin fusion proteins of the invention may be fused to the *pelB* pectate lyase signal sequence to increase the efficiency of expression and purification of such polypeptides in Gram-negative bacteria. See, U.S. Patent Nos. 5,576,195 and 5,846,818, the contents of which are herein incorporated by reference in their entireties.

Examples of signal peptides that may be fused to an albumin fusion protein of the invention in order to direct its secretion in mammalian cells include, but are not limited to, the MPIF-1 signal sequence (e.g., amino acids 1-21 of GenBank Accession number

AAB51134), the stanniocalcin signal sequence (MLQNSAVLLLLVISASA, SEQ ID NO:34), and a consensus signal sequence (MPTWAWWLFLVLLLALWAPARG, SEQ ID NO:35). A suitable signal sequence that may be used in conjunction with baculoviral expression systems is the gp67 signal sequence (e.g., amino acids 1-19 of GenBank Accession Number AAA72759).

Vectors which use glutamine synthase (GS) or DHFR as the selectable markers can be amplified in the presence of the drugs methionine sulphoximine or methotrexate, respectively. An advantage of glutamine synthase based vectors are the availability of cell lines (e.g., the murine myeloma cell line, NSO) which are glutamine synthase negative.

Glutamine synthase expression systems can also function in glutamine synthase expressing cells (e.g., Chinese Hamster Ovary (CHO) cells) by providing additional inhibitor to prevent the functioning of the endogenous gene. A glutamine synthase expression system and components thereof are detailed in PCT publications: WO87/04462; WO86/05807; WO89/01036; WO89/10404; and WO91/06657, which are hereby incorporated in their entireties by reference herein. Additionally, glutamine synthase expression vectors can be obtained from Lonza Biologics, Inc. (Portsmouth, NH). Expression and production of monoclonal antibodies using a GS expression system in murine myeloma cells is described in Bebbington *et al.*, *Bio/technology* 10:169(1992) and in Biblia and Robinson *Biotechnol. Prog.* 11:1 (1995) which are herein incorporated by reference.

The present invention also relates to host cells containing the above-described vector constructs described herein, and additionally encompasses host cells containing nucleotide sequences of the invention that are operably associated with one or more heterologous control regions (e.g., promoter and/or enhancer) using techniques known of in the art. The host cell can be a higher eukaryotic cell, such as a mammalian cell (e.g., a human derived cell), or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. A host strain may be chosen which modulates the expression of the inserted gene sequences, or modifies and processes the gene product in the specific fashion desired. Expression from certain promoters can be elevated in the presence of certain inducers; thus expression of the genetically engineered polypeptide may be controlled. Furthermore, different host cells have characteristics and specific mechanisms for the translational and post-translational processing and modification (e.g., phosphorylation, cleavage) of proteins. Appropriate cell lines can be chosen to ensure the

desired modifications and processing of the foreign protein expressed.

Introduction of the nucleic acids and nucleic acid constructs of the invention into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection, electroporation, transduction, infection, or other methods. Such methods are described in many standard laboratory manuals, such as Davis et al., Basic Methods In Molecular Biology (1986). It is specifically contemplated that the polypeptides of the present invention may in fact be expressed by a host cell lacking a recombinant vector.

In addition to encompassing host cells containing the vector constructs discussed herein, the invention also encompasses primary, secondary, and immortalized host cells of vertebrate origin, particularly mammalian origin, that have been engineered to delete or replace endogenous genetic material (e.g., the coding sequence corresponding to a Therapeutic protein may be replaced with an albumin fusion protein corresponding to the Therapeutic protein), and/or to include genetic material (e.g., heterologous polynucleotide sequences such as for example, an albumin fusion protein of the invention corresponding to the Therapeutic protein may be included). The genetic material operably associated with the endogenous polynucleotide may activate, alter, and/or amplify endogenous polynucleotides.

In addition, techniques known in the art may be used to operably associate heterologous polynucleotides (e.g., polynucleotides encoding an albumin protein, or a fragment or variant thereof) and/or heterologous control regions (e.g., promoter and/or enhancer) with endogenous polynucleotide sequences encoding a Therapeutic protein via homologous recombination (see, e.g., US Patent Number 5,641,670, issued June 24, 1997; International Publication Number WO 96/29411; International Publication Number WO 94/12650; Koller et al., *Proc. Natl. Acad. Sci. USA* 86:8932-8935 (1989); and Zijlstra et al., *Nature* 342:435-438 (1989), the disclosures of each of which are incorporated by reference in their entireties).

Albumin fusion proteins of the invention can be recovered and purified from recombinant cell cultures by well-known methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography, hydrophobic charge interaction chromatography and

lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification.

In preferred embodiments the albumin fusion proteins of the invention are purified using Anion Exchange Chromatography including, but not limited to, chromatography on  
5 Q-sepharose, DEAE sepharose, poros HQ, poros DEAE, Toyopearl Q, Toyopearl QAE, Toyopearl DEAE, Resource/Source Q and DEAE, Fractogel Q and DEAE columns.

In specific embodiments the albumin fusion proteins of the invention are purified using Cation Exchange Chromatography including, but not limited to, SP-sepharose, CM  
10 sepharose, poros HS, poros CM, Toyopearl SP, Toyopearl CM, Resource/Source S and CM, Fractogel S and CM columns and their equivalents and comparables.

In specific embodiments the albumin fusion proteins of the invention are purified using Hydrophobic Interaction Chromatography including, but not limited to, Phenyl,  
Butyl, Methyl, Octyl, Hexyl-sepharose, poros Phenyl, Butyl, Methyl, Octyl, Hexyl ,  
15 Toyopearl Phenyl, Butyl, Methyl, Octyl, Hexyl Resource/Source Phenyl, Butyl, Methyl, Octyl, Hexyl, Fractogel Phenyl, Butyl, Methyl, Octyl, Hexyl columns and their equivalents and comparables.

In specific embodiments the albumin fusion proteins of the invention are purified using Size Exclusion Chromatography including, but not limited to, sepharose S100,  
S200, S300, superdex resin columns and their equivalents and comparables.

20 In specific embodiments the albumin fusion proteins of the invention are purified using Affinity Chromatography including, but not limited to, Mimetic Dye affinity, peptide affinity and antibody affinity columns that are selective for either the HSA or the "fusion target" molecules.

In preferred embodiments albumin fusion proteins of the invention are purified  
25 using one or more Chromatography methods listed above. In other preferred embodiments, albumin fusion proteins of the invention are purified using one or more of the following Chromatography columns, Q sepharose FF column, SP Sepharose FF column, Q Sepharose High Performance Column, Blue Sepharose FF column , Blue Column, Phenyl Sepharose FF column, DEAE Sepharose FF, or Methyl Column.

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Additionally, albumin fusion proteins of the invention may be purified using the process described in PCT International Publication WO 00/44772 which is herein incorporated by reference in its entirety. One of skill in the art could easily modify the process described therein for use in the purification of albumin fusion proteins of the invention.

Albumin fusion proteins of the present invention may be recovered from: products of chemical synthetic procedures; and products produced by recombinant techniques from a prokaryotic or eukaryotic host, including, for example, bacterial, yeast, higher plant, insect, and mammalian cells. Depending upon the host employed in a recombinant production procedure, the polypeptides of the present invention may be glycosylated or may be non-glycosylated. In addition, albumin fusion proteins of the invention may also include an initial modified methionine residue, in some cases as a result of host-mediated processes. Thus, it is well known in the art that the N-terminal methionine encoded by the translation initiation codon generally is removed with high efficiency from any protein after translation in all eukaryotic cells. While the N-terminal methionine on most proteins also is efficiently removed in most prokaryotes, for some proteins, this prokaryotic removal process is inefficient, depending on the nature of the amino acid to which the N-terminal methionine is covalently linked.

In one embodiment, the yeast *Pichia pastoris* is used to express albumin fusion proteins of the invention in a eukaryotic system. *Pichia pastoris* is a methylotrophic yeast which can metabolize methanol as its sole carbon source. A main step in the methanol metabolism pathway is the oxidation of methanol to formaldehyde using O<sub>2</sub>. This reaction is catalyzed by the enzyme alcohol oxidase. In order to metabolize methanol as its sole carbon source, *Pichia pastoris* must generate high levels of alcohol oxidase due, in part, to the relatively low affinity of alcohol oxidase for O<sub>2</sub>. Consequently, in a growth medium depending on methanol as a main carbon source, the promoter region of one of the two alcohol oxidase genes (*AOX1*) is highly active. In the presence of methanol, alcohol oxidase produced from the *AOX1* gene comprises up to approximately 30% of the total soluble protein in *Pichia pastoris*. See Ellis, S.B., et al., *Mol. Cell. Biol.* 5:1111-21 (1985); Koutz, P.J., et al., *Yeast* 5:167-77 (1989); Tschopp, J.F., et al., *Nucl. Acids Res.* 15:3859-76 (1987). Thus, a heterologous coding sequence, such as, for example, a



polynucleotide of the present invention, under the transcriptional regulation of all or part of the *AOX1* regulatory sequence is expressed at exceptionally high levels in *Pichia* yeast grown in the presence of methanol.

In one example, the plasmid vector pPIC9K is used to express DNA encoding an albumin fusion protein of the invention, as set forth herein, in a *Pichea* yeast system essentially as described in "*Pichia* Protocols: Methods in Molecular Biology," D.R. Higgins and J. Cregg, eds. The Humana Press, Totowa, NJ, 1998. This expression vector allows expression and secretion of a polypeptide of the invention by virtue of the strong *AOX1* promoter linked to the *Pichia pastoris* alkaline phosphatase (PHO) secretory signal peptide (i.e., leader) located upstream of a multiple cloning site.

Many other yeast vectors could be used in place of pPIC9K, such as, pYES2, pYD1, pTEF1/Zeo, pYES2/GS, pPICZ, pGAPZ, pGAPZalpha, pPIC9, pPIC3.5, pHIL-D2, pHIL-S1, pPIC3.5K, and PAO815, as one skilled in the art would readily appreciate, as long as the proposed expression construct provides appropriately located signals for transcription, translation, secretion (if desired), and the like, including an in-frame AUG as required.

In another embodiment, high-level expression of a heterologous coding sequence, such as, for example, a polynucleotide encoding an albumin fusion protein of the present invention, may be achieved by cloning the heterologous polynucleotide of the invention into an expression vector such as, for example, pGAPZ or pGAPZalpha, and growing the yeast culture in the absence of methanol.

In addition, albumin fusion proteins of the invention can be chemically synthesized using techniques known in the art (e.g., see Creighton, 1983, *Proteins: Structures and Molecular Principles*, W.H. Freeman & Co., N.Y., and Hunkapiller et al., *Nature*, 310:105-111 (1984)). For example, a polypeptide corresponding to a fragment of a polypeptide can be synthesized by use of a peptide synthesizer. Furthermore, if desired, nonclassical amino acids or chemical amino acid analogs can be introduced as a substitution or addition into the polypeptide sequence. Non-classical amino acids include, but are not limited to, to the D-isomers of the common amino acids, 2,4-diaminobutyric acid,  $\alpha$ -amino isobutyric acid, 4-aminobutyric acid, Abu, 2-amino butyric acid, g-Abu, e-Ahx, 6-amino hexanoic acid, Aib, 2-amino isobutyric acid, 3-amino propionic acid,

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ornithine, norleucine, norvaline, hydroxyproline, sarcosine, citrulline, homocitrulline, cysteic acid, t-butylglycine, t-butylalanine, phenylglycine, cyclohexylalanine, b-alanine, fluoro-amino acids, designer amino acids such as b-methyl amino acids, Ca-methyl amino acids, Na-methyl amino acids, and amino acid analogs in general. Furthermore, the amino acid can be D (dextrorotary) or L (levorotary).

The invention encompasses albumin fusion proteins of the present invention which are differentially modified during or after translation, e.g., by glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to an antibody molecule or other cellular ligand, etc. Any of numerous chemical modifications may be carried out by known techniques, including but not limited, to specific chemical cleavage by cyanogen bromide, trypsin, chymotrypsin, papain, V8 protease, NaBH<sub>4</sub>; acetylation, formylation, oxidation, reduction; metabolic synthesis in the presence of tunicamycin; etc.

Additional post-translational modifications encompassed by the invention include, for example, e.g., N-linked or O-linked carbohydrate chains, processing of N-terminal or C-terminal ends), attachment of chemical moieties to the amino acid backbone, chemical modifications of N-linked or O-linked carbohydrate chains, and addition or deletion of an N-terminal methionine residue as a result of procaryotic host cell expression. The albumin fusion proteins may also be modified with a detectable label, such as an enzymatic, fluorescent, isotopic or affinity label to allow for detection and isolation of the protein.

Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include iodine (<sup>121</sup>I, <sup>123</sup>I, <sup>125</sup>I, <sup>131</sup>I), carbon (<sup>14</sup>C), sulfur (<sup>35</sup>S), tritium (<sup>3</sup>H), indium (<sup>111</sup>In, <sup>112</sup>In, <sup>113m</sup>In, <sup>115m</sup>In), technetium (<sup>99</sup>Tc, <sup>99m</sup>Tc), thallium (<sup>201</sup>Tl), gallium (<sup>68</sup>Ga, <sup>67</sup>Ga), palladium (<sup>103</sup>Pd), molybdenum (<sup>99</sup>Mo), xenon (<sup>133</sup>Xe), fluorine (<sup>18</sup>F), <sup>153</sup>Sm, <sup>177</sup>Lu, <sup>159</sup>Gd, <sup>149</sup>Pm, <sup>140</sup>La, <sup>175</sup>Yb, <sup>166</sup>Ho, <sup>90</sup>Y, <sup>47</sup>Sc, <sup>186</sup>Re, <sup>188</sup>Re, <sup>142</sup>Pr, <sup>105</sup>Rh, and <sup>97</sup>Ru.

In specific embodiments, albumin fusion proteins of the present invention or

fragments or variants thereof are attached to macrocyclic chelators that associate with radiometal ions, including but not limited to, <sup>177</sup>Lu, <sup>90</sup>Y, <sup>166</sup>Ho, and <sup>153</sup>Sm, to polypeptides.

In a preferred embodiment, the radiometal ion associated with the macrocyclic chelators is <sup>111</sup>In. In another preferred embodiment, the radiometal ion associated with the

5 macrocyclic chelator is <sup>90</sup>Y. In specific embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA). In other specific embodiments, DOTA is attached to an antibody of the invention or fragment thereof via linker molecule. Examples of linker molecules useful for conjugating DOTA to a polypeptide are commonly known in the art - see, for example, DeNardo et al., Clin  
10 Cancer Res. 4(10):2483-90 (1998); Peterson et al., Bioconjug. Chem. 10(4):553-7 (1999); and Zimmerman et al, Nucl. Med. Biol. 26(8):943-50 (1999); which are hereby incorporated by reference in their entirety.

As mentioned, the albumin fusion proteins of the invention may be modified by either natural processes, such as post-translational processing, or by chemical modification  
15 techniques which are well known in the art. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given polypeptide. Polypeptides of the invention may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic polypeptides may result from posttranslation natural processes or may be  
20 made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links,  
25 formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS -  
30 STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POST-TRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs.

1-12 (1983); Seifter et al., Meth. Enzymol. 182:626-646 (1990); Rattan et al., Ann. N.Y. Acad. Sci. 663:48-62 (1992)).

Albumin fusion proteins of the invention and antibodies that bind a Therapeutic protein or fragments or variants thereof can be fused to marker sequences, such as a peptide to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz et al., Proc. Natl. Acad. Sci. USA 86:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson et al., Cell 37:767 (1984)) and the "flag" tag.

Further, an albumin fusion protein of the invention may be conjugated to a therapeutic moiety such as a cytotoxin, e.g., a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example, <sup>213</sup>Bi. A cytotoxin or cytotoxic agent includes any agent that is detrimental to cells. Examples include paclitaxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents include, but are not limited to, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis- dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (e.g., vincristine and vinblastine).

The conjugates of the invention can be used for modifying a given biological response, the therapeutic agent or drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or

polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein such as tumor necrosis factor, alpha-interferon, beta-interferon, nerve growth factor, platelet derived growth factor, tissue plasminogen activator, an apoptotic agent, e.g., TNF-alpha, TNF-beta, AIM I (See, International Publication No. WO 97/33899), AIM II (See, International Publication No. WO 97/34911), Fas Ligand (Takahashi *et al.*, *Int. Immunol.*, 6:1567-1574 (1994)), VEGI (See, International Publication No. WO 99/23105), a thrombotic agent or an anti-angiogenic agent, e.g., angiostatin or endostatin; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors. Techniques for conjugating such therapeutic moiety to proteins (e.g., albumin fusion proteins) are well known in the art.

Albumin fusion proteins may also be attached to solid supports, which are particularly useful for immunoassays or purification of polypeptides that are bound by, that bind to, or associate with albumin fusion proteins of the invention. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

Albumin fusion proteins, with or without a therapeutic moiety conjugated to it, administered alone or in combination with cytotoxic factor(s) and/or cytokine(s) can be used as a therapeutic.

In embodiments where the albumin fusion protein of the invention comprises only the VH domain of an antibody that binds a Therapeutic protein, it may be necessary and/or desirable to coexpress the fusion protein with the VL domain of the same antibody that binds a Therapeutic protein, such that the VH-albumin fusion protein and VL protein will associate (either covalently or non-covalently) post-translationally.

In embodiments where the albumin fusion protein of the invention comprises only the VL domain of an antibody that binds a Therapeutic protein, it may be necessary and/or desirable to coexpress the fusion protein with the VH domain of the same antibody that binds a Therapeutic protein, such that the VL-albumin fusion protein and VH protein will associate (either covalently or non-covalently) post-translationally.

Some Therapeutic antibodies are bispecific antibodies, meaning the antibody that

binds a Therapeutic protein is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. In order to create an albumin fusion protein corresponding to that Therapeutic protein, it is possible to create an albumin fusion protein which has an scFv fragment fused to both the N- and C- terminus of the albumin protein moiety. More particularly, the scFv fused to the N-terminus of albumin would correspond to one of the heavy/light (VH/VL) pairs of the original antibody that binds a Therapeutic protein and the scFv fused to the C-terminus of albumin would correspond to the other heavy/light (VH/VL) pair of the original antibody that binds a Therapeutic protein.

Also provided by the invention are chemically modified derivatives of the albumin fusion proteins of the invention which may provide additional advantages such as increased solubility, stability and circulating time of the polypeptide, or decreased immunogenicity (see U.S. Patent No. 4,179,337). The chemical moieties for derivitization may be selected from water soluble polymers such as polyethylene glycol, ethylene glycol/propylene glycol copolymers, carboxymethylcellulose, dextran, polyvinyl alcohol and the like. The albumin fusion proteins may be modified at random positions within the molecule, or at predetermined positions within the molecule and may include one, two, three or more attached chemical moieties.

The polymer may be of any molecular weight, and may be branched or unbranched. For polyethylene glycol, the preferred molecular weight is between about 1 kDa and about 100 kDa (the term "about" indicating that in preparations of polyethylene glycol, some molecules will weigh more, some less, than the stated molecular weight) for ease in handling and manufacturing. Other sizes may be used, depending on the desired therapeutic profile (e.g., the duration of sustained release desired, the effects, if any on biological activity, the ease in handling, the degree or lack of antigenicity and other known effects of the polyethylene glycol to a Therapeutic protein or analog). For example, the polyethylene glycol may have an average molecular weight of about 200, 500, 1000, 1500, 2000, 2500, 3000, 3500, 4000, 4500, 5000, 5500, 6000, 6500, 7000, 7500, 8000, 8500, 9000, 9500, 10,000, 10,500, 11,000, 11,500, 12,000, 12,500, 13,000, 13,500, 14,000, 14,500, 15,000, 15,500, 16,000, 16,500, 17,000, 17,500, 18,000, 18,500, 19,000, 19,500, 20,000, 25,000, 30,000, 35,000, 40,000, 45,000, 50,000, 55,000, 60,000, 65,000, 70,000, 75,000, 80,000, 85,000, 90,000, 95,000, or 100,000 kDa.

As noted above, the polyethylene glycol may have a branched structure. Branched polyethylene glycols are described, for example, in U.S. Patent No. 5,643,575; Morpurgo *et al.*, *Appl. Biochem. Biotechnol.* 56:59-72 (1996); Vorobjev *et al.*, *Nucleosides Nucleotides* 18:2745-2750 (1999); and Caliceti *et al.*, *Bioconjug. Chem.* 10:638-646 (1999), the disclosures of each of which are incorporated herein by reference.

The polyethylene glycol molecules (or other chemical moieties) should be attached to the protein with consideration of effects on functional or antigenic domains of the protein. There are a number of attachment methods available to those skilled in the art, such as, for example, the method disclosed in EP 0 401 384 (coupling PEG to G-CSF), herein incorporated by reference; see also Malik *et al.*, *Exp. Hematol.* 20:1028-1035 (1992), reporting pegylation of GM-CSF using tresyl chloride. For example, polyethylene glycol may be covalently bound through amino acid residues via reactive group, such as a free amino or carboxyl group. Reactive groups are those to which an activated polyethylene glycol molecule may be bound. The amino acid residues having a free amino group may include lysine residues and the N-terminal amino acid residues; those having a free carboxyl group may include aspartic acid residues glutamic acid residues and the C-terminal amino acid residue. Sulfhydryl groups may also be used as a reactive group for attaching the polyethylene glycol molecules. Preferred for therapeutic purposes is attachment at an amino group, such as attachment at the N-terminus or lysine group.

As suggested above, polyethylene glycol may be attached to proteins via linkage to any of a number of amino acid residues. For example, polyethylene glycol can be linked to proteins via covalent bonds to lysine, histidine, aspartic acid, glutamic acid, or cysteine residues. One or more reaction chemistries may be employed to attach polyethylene glycol to specific amino acid residues (e.g., lysine, histidine, aspartic acid, glutamic acid, or cysteine) of the protein or to more than one type of amino acid residue (e.g., lysine, histidine, aspartic acid, glutamic acid, cysteine and combinations thereof) of the protein.

One may specifically desire proteins chemically modified at the N-terminus. Using polyethylene glycol as an illustration of the present composition, one may select from a variety of polyethylene glycol molecules (by molecular weight, branching, etc.), the proportion of polyethylene glycol molecules to protein (polypeptide) molecules in the reaction mix, the type of pegylation reaction to be performed, and the method of obtaining the selected N-terminally pegylated protein. The method of obtaining the N-terminally

pegylated preparation (i.e., separating this moiety from other monopegylated moieties if necessary) may be by purification of the N-terminally pegylated material from a population of pegylated protein molecules. Selective proteins chemically modified at the N-terminus modification may be accomplished by reductive alkylation which exploits differential reactivity of different types of primary amino groups (lysine versus the N-terminal) available for derivatization in a particular protein. Under the appropriate reaction conditions, substantially selective derivatization of the protein at the N-terminus with a carbonyl group containing polymer is achieved.

As indicated above, pegylation of the albumin fusion proteins of the invention may be accomplished by any number of means. For example, polyethylene glycol may be attached to the albumin fusion protein either directly or by an intervening linker. Linkerless systems for attaching polyethylene glycol to proteins are described in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992); Francis et al., Intern. J. of Hematol. 68:1-18 (1998); U.S. Patent No. 4,002,531; U.S. Patent No. 5,349,052; WO 95/06058; and WO 98/32466, the disclosures of each of which are incorporated herein by reference.

One system for attaching polyethylene glycol directly to amino acid residues of proteins without an intervening linker employs tresylated MPEG, which is produced by the modification of monmethoxy polyethylene glycol (MPEG) using tresylchloride ( $\text{ClSO}_2\text{CH}_2\text{CF}_3$ ). Upon reaction of protein with tresylated MPEG, polyethylene glycol is directly attached to amine groups of the protein. Thus, the invention includes protein-polyethylene glycol conjugates produced by reacting proteins of the invention with a polyethylene glycol molecule having a 2,2,2-trifluoroethane sulphonyl group.

Polyethylene glycol can also be attached to proteins using a number of different intervening linkers. For example, U.S. Patent No. 5,612,460, the entire disclosure of which is incorporated herein by reference, discloses urethane linkers for connecting polyethylene glycol to proteins. Protein-polyethylene glycol conjugates wherein the polyethylene glycol is attached to the protein by a linker can also be produced by reaction of proteins with compounds such as MPEG-succinimidylsuccinate, MPEG activated with 1,1'-carbonyldiimidazole, MPEG-2,4,5-trichloropenylcarbonate, MPEG-p-nitrophenolcarbonate, and various MPEG-succinate derivatives. A number of additional polyethylene glycol derivatives and reaction chemistries for attaching polyethylene glycol



to proteins are described in International Publication No. WO 98/32466, the entire disclosure of which is incorporated herein by reference. Pegylated protein products produced using the reaction chemistries set out herein are included within the scope of the invention.

5           The number of polyethylene glycol moieties attached to each albumin fusion protein of the invention (i.e., the degree of substitution) may also vary. For example, the pegylated proteins of the invention may be linked, on average, to 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 15, 17, 20, or more polyethylene glycol molecules. Similarly, the average degree of substitution within ranges such as 1-3, 2-4, 3-5, 4-6, 5-7, 6-8, 7-9, 8-10, 9-11, 10-12, 10 11-13, 12-14, 13-15, 14-16, 15-17, 16-18, 17-19, or 18-20 polyethylene glycol moieties per protein molecule. Methods for determining the degree of substitution are discussed, for example, in Delgado et al., Crit. Rev. Thera. Drug Carrier Sys. 9:249-304 (1992).

          The polypeptides of the invention can be recovered and purified from chemical synthesis and recombinant cell cultures by standard methods which include, but are not 15 limited to, ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Most preferably, high performance liquid chromatography ("HPLC") is employed for purification. Well known techniques for refolding protein may be employed 20 to regenerate active conformation when the polypeptide is denatured during isolation and/or purification.

5 The presence and quantity of albumin fusion proteins of the invention may be determined using ELISA, a well known immunoassay known in the art. In one ELISA protocol that would be useful for detecting/quantifying albumin fusion proteins of the invention, comprises the steps of coating an ELISA plate with an anti-human serum albumin antibody, blocking the plate to prevent non-specific binding, washing the ELISA plate, adding a solution containing the albumin fusion protein of the invention (at one or more different concentrations), adding a secondary anti-Therapeutic protein specific antibody coupled to a detectable label (as described herein or otherwise known in the art), and detecting the presence of the secondary antibody. In an alternate version of this protocol, the ELISA plate might be coated with the anti-Therapeutic protein specific antibody and the labeled secondary reagent might be the anti-human albumin specific antibody.

#### Uses of the Polynucleotides

15 Each of the polynucleotides identified herein can be used in numerous ways as reagents. The following description should be considered exemplary and utilizes known techniques.

20 The polynucleotides of the present invention are useful to produce the albumin fusion proteins of the invention. As described in more detail below, polynucleotides of the invention (encoding albumin fusion proteins) may be used in recombinant DNA methods useful in genetic engineering to make cells, cell lines, or tissues that express the albumin fusion protein encoded by the polynucleotides encoding albumin fusion proteins of the invention.

25 Polynucleotides of the present invention are also useful in gene therapy. One goal of gene therapy is to insert a normal gene into an organism having a defective gene, in an effort to correct the genetic defect. The polynucleotides disclosed in the present invention offer a means of targeting such genetic defects in a highly accurate manner. Another goal is to insert a new gene that was not present in the host genome, thereby producing a new trait in the host cell. Additional non-limiting examples of gene therapy methods encompassed by the present invention are more thoroughly described elsewhere herein (see, e.g., the sections labeled "Gene Therapy", and Examples 17 and 18).

## Uses of the Polypeptides

Each of the polypeptides identified herein can be used in numerous ways. The following description should be considered exemplary and utilizes known techniques.

Albumin fusion proteins of the invention are useful to provide immunological probes for differential identification of the tissue(s) (e.g., immunohistochemistry assays such as, for example, ABC immunoperoxidase (Hsu et al., J. Histochem. Cytochem. 29:577-580 (1981)) or cell type(s) (e.g., immunocytochemistry assays).

Albumin fusion proteins can be used to assay levels of polypeptides in a biological sample using classical immunohistological methods known to those of skill in the art (e.g., see Jalkanen, et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, et al., J. Cell. Biol. 105:3087-3096 (1987)). Other methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable assay labels are known in the art and include enzyme labels, such as, glucose oxidase; radioisotopes, such as iodine ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ),  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,  $^{47}\text{Sc}$ ,  $^{186}\text{Re}$ ,  $^{188}\text{Re}$ ,  $^{142}\text{Pr}$ ,  $^{105}\text{Rh}$ ,  $^{97}\text{Ru}$ ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin.

Albumin fusion proteins of the invention can also be detected *in vivo* by imaging. Labels or markers for *in vivo* imaging of protein include those detectable by X-radiography, nuclear magnetic resonance (NMR) or electron spin relaxation (ESR). For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the albumin fusion protein by labeling of nutrients given to a cell line expressing the albumin fusion protein of the invention.

An albumin fusion protein which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ , ( $^{131}\text{I}$ ,  $^{125}\text{I}$ ,  $^{123}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{115\text{m}}\text{In}$ ,  $^{113\text{m}}\text{In}$ ,  $^{112}\text{In}$ ,  $^{111}\text{In}$ ), and technetium ( $^{99}\text{Tc}$ ,  $^{99\text{m}}\text{Tc}$ ), thallium ( $^{201}\text{Tl}$ ), gallium ( $^{68}\text{Ga}$ ,  $^{67}\text{Ga}$ ), palladium ( $^{103}\text{Pd}$ ), molybdenum ( $^{99}\text{Mo}$ ), xenon ( $^{133}\text{Xe}$ ), fluorine ( $^{18}\text{F}$ ,  $^{153}\text{Sm}$ ,  $^{177}\text{Lu}$ ,  $^{159}\text{Gd}$ ,  $^{149}\text{Pm}$ ,  $^{140}\text{La}$ ,  $^{175}\text{Yb}$ ,  $^{166}\text{Ho}$ ,  $^{90}\text{Y}$ ,

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<sup>47</sup>Sc, <sup>186</sup>Re, <sup>188</sup>Re, <sup>142</sup>Pr, <sup>105</sup>Rh, <sup>97</sup>Ru), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for immune system disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of <sup>99m</sup>Tc. The labeled albumin fusion protein will then preferentially accumulate at locations in the body (e.g., organs, cells, extracellular spaces or matrices) where one or more receptors, ligands or substrates (corresponding to that of the Therapeutic protein used to make the albumin fusion protein of the invention) are located. Alternatively, in the case where the albumin fusion protein comprises at least a fragment or variant of a Therapeutic antibody, the labeled albumin fusion protein will then preferentially accumulate at the locations in the body (e.g., organs, cells, extracellular spaces or matrices) where the polypeptides/epitopes corresponding to those bound by the Therapeutic antibody (used to make the albumin fusion protein of the invention) are located. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)). The protocols described therein could easily be modified by one of skill in the art for use with the albumin fusion proteins of the invention.

In one embodiment, the invention provides a method for the specific delivery of albumin fusion proteins of the invention to cells by administering albumin fusion proteins of the invention (e.g., polypeptides encoded by polynucleotides encoding albumin fusion proteins of the invention and/or antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a Therapeutic protein into the targeted cell. In another example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering albumin fusion

proteins of the invention in association with toxins or cytotoxic prodrugs.

By "toxin" is meant one or more compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. "Toxin" also includes a cytostatic or cytocidal agent, a therapeutic agent or a radioactive metal ion, e.g., alpha-emitters such as, for example,  $^{213}\text{Bi}$ , or other radioisotopes such as, for example,  $^{103}\text{Pd}$ ,  $^{133}\text{Xe}$ ,  $^{131}\text{I}$ ,  $^{68}\text{Ge}$ ,  $^{57}\text{Co}$ ,  $^{65}\text{Zn}$ ,  $^{85}\text{Sr}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{153}\text{Sm}$ ,  $^{153}\text{Gd}$ ,  $^{169}\text{Yb}$ ,  $^{51}\text{Cr}$ ,  $^{54}\text{Mn}$ ,  $^{75}\text{Se}$ ,  $^{113}\text{Sn}$ ,  $^{90}\text{Yttrium}$ ,  $^{117}\text{Tin}$ ,  $^{186}\text{Rhenium}$ ,  $^{166}\text{Holmium}$ , and  $^{188}\text{Rhenium}$ ; luminescent labels, such as luminol; and fluorescent labels, such as fluorescein and rhodamine, and biotin. In a specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope  $^{90}\text{Y}$ . In another specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope  $^{111}\text{In}$ . In a further specific embodiment, the invention provides a method for the specific destruction of cells (e.g., the destruction of tumor cells) by administering polypeptides of the invention or antibodies of the invention in association with the radioisotope  $^{131}\text{I}$ .

Techniques known in the art may be applied to label polypeptides of the invention. Such techniques include, but are not limited to, the use of bifunctional conjugating agents (see e.g., U.S. Patent Nos. 5,756,065; 5,714,631; 5,696,239; 5,652,361; 5,505,931; 5,489,425; 5,435,990; 5,428,139; 5,342,604; 5,274,119; 4,994,560; and 5,808,003; the contents of each of which are hereby incorporated by reference in its entirety).

The albumin fusion proteins of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably

humans. Such disorders include, but are not limited to, those described herein under the section heading "Biological Activities," below.

Thus, the invention provides a diagnostic method of a disorder, which involves (a) assaying the expression level of a certain polypeptide in cells or body fluid of an individual using an albumin fusion protein of the invention; and (b) comparing the assayed polypeptide expression level with a standard polypeptide expression level, whereby an increase or decrease in the assayed polypeptide expression level compared to the standard expression level is indicative of a disorder. With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Moreover, albumin fusion proteins of the present invention can be used to treat or prevent diseases or conditions such as, for example, neural disorders, immune system disorders, muscular disorders, reproductive disorders, gastrointestinal disorders, pulmonary disorders, cardiovascular disorders, renal disorders, proliferative disorders, and/or cancerous diseases and conditions. For example, patients can be administered a polypeptide of the present invention in an effort to replace absent or decreased levels of the polypeptide (e.g., insulin), to supplement absent or decreased levels of a different polypeptide (e.g., hemoglobin S for hemoglobin B, SOD, catalase, DNA repair proteins), to inhibit the activity of a polypeptide (e.g., an oncogene or tumor suppressor), to activate the activity of a polypeptide (e.g., by binding to a receptor), to reduce the activity of a membrane bound receptor by competing with it for free ligand (e.g., soluble TNF receptors used in reducing inflammation), or to bring about a desired response (e.g., blood vessel growth inhibition, enhancement of the immune response to proliferative cells or tissues).

In particular, albumin fusion proteins comprising of at least a fragment or variant of a Therapeutic antibody can also be used to treat disease (as described *supra*, and elsewhere herein). For example, administration of an albumin fusion protein comprising of at least a fragment or variant of a Therapeutic antibody can bind, and/or neutralize the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein

specifically binds, and/or reduce overproduction of the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds. Similarly, administration of an albumin fusion protein comprising of at least a fragment or variant of a Therapeutic antibody can activate the polypeptide to which the Therapeutic antibody used to make the albumin fusion protein specifically binds, by binding to the polypeptide bound to a membrane (receptor).

At the very least, the albumin fusion proteins of the invention of the present invention can be used as molecular weight markers on SDS-PAGE gels or on molecular sieve gel filtration columns using methods well known to those of skill in the art.

Albumin fusion proteins of the invention can also be used to raise antibodies, which in turn may be used to measure protein expression of the Therapeutic protein, albumin protein, and/or the albumin fusion protein of the invention from a recombinant cell, as a way of assessing transformation of the host cell, or in a biological sample. Moreover, the albumin fusion proteins of the present invention can be used to test the biological activities described herein.

#### ***Diagnostic Assays***

The compounds of the present invention are useful for diagnosis, treatment, prevention and/or prognosis of various disorders in mammals, preferably humans. Such disorders include, but are not limited to, those described for each Therapeutic protein in the corresponding row of Table 1 and herein under the section headings "Immune Activity," "Blood Related Disorders," "Hyperproliferative Disorders," "Renal Disorders," "Cardiovascular Disorders," "Respiratory Disorders," "Anti-Angiogenesis Activity," "Diseases at the Cellular Level," "Wound Healing and Epithelial Cell Proliferation," "Neural Activity and Neurological Diseases," "Endocrine Disorders," "Reproductive System Disorders," "Infectious Disease," "Regeneration," and/or "Gastrointestinal Disorders," *infra*.

For a number of disorders, substantially altered (increased or decreased) levels of gene expression can be detected in tissues, cells or bodily fluids (e.g., sera, plasma, urine, semen, synovial fluid or spinal fluid) taken from an individual having such a disorder, relative to a "standard" gene expression level, that is, the expression level in tissues or bodily fluids from an individual not having the disorder. Thus, the invention provides a

diagnostic method useful during diagnosis of a disorder, which involves measuring the expression level of the gene encoding a polypeptide in tissues, cells or body fluid from an individual and comparing the measured gene expression level with a standard gene expression level, whereby an increase or decrease in the gene expression level(s) compared to the standard is indicative of a disorder. These diagnostic assays may be performed *in vivo* or *in vitro*, such as, for example, on blood samples, biopsy tissue or autopsy tissue.

The present invention is also useful as a prognostic indicator, whereby patients exhibiting enhanced or depressed gene expression will experience a worse clinical outcome

By "assaying the expression level of the gene encoding a polypeptide" is intended qualitatively or quantitatively measuring or estimating the level of a particular polypeptide (e.g. a polypeptide corresponding to a Therapeutic protein disclosed in Table 1) or the level of the mRNA encoding the polypeptide of the invention in a first biological sample either directly (e.g., by determining or estimating absolute protein level or mRNA level) or relatively (e.g., by comparing to the polypeptide level or mRNA level in a second biological sample). Preferably, the polypeptide expression level or mRNA level in the first biological sample is measured or estimated and compared to a standard polypeptide level or mRNA level, the standard being taken from a second biological sample obtained from an individual not having the disorder or being determined by averaging levels from a population of individuals not having the disorder. As will be appreciated in the art, once a standard polypeptide level or mRNA level is known, it can be used repeatedly as a standard for comparison.

By "biological sample" is intended any biological sample obtained from an individual, cell line, tissue culture, or other source containing polypeptides of the invention (including portions thereof) or mRNA. As indicated, biological samples include body fluids (such as sera, plasma, urine, synovial fluid and spinal fluid) and tissue sources found to express the full length or fragments thereof of a polypeptide or mRNA. Methods for obtaining tissue biopsies and body fluids from mammals are well known in the art. Where the biological sample is to include mRNA, a tissue biopsy is the preferred source.

Total cellular RNA can be isolated from a biological sample using any suitable technique such as the single-step guanidinium-thiocyanate-phenol-chloroform method



described in Chomczynski and Sacchi, Anal. Biochem. 162:156-159 (1987). Levels of mRNA encoding the polypeptides of the invention are then assayed using any appropriate method. These include Northern blot analysis, S1 nuclease mapping, the polymerase chain reaction (PCR), reverse transcription in combination with the polymerase chain reaction (RT-PCR), and reverse transcription in combination with the ligase chain reaction (RT-LCR).

The present invention also relates to diagnostic assays such as quantitative and diagnostic assays for detecting levels of polypeptides that bind to, are bound by, or associate with albumin fusion proteins of the invention, in a biological sample (e.g., cells and tissues), including determination of normal and abnormal levels of polypeptides. Thus, for instance, a diagnostic assay in accordance with the invention for detecting abnormal expression of polypeptides that bind to, are bound by, or associate with albumin fusion proteins compared to normal control tissue samples may be used to detect the presence of tumors. Assay techniques that can be used to determine levels of a polypeptide that bind to, are bound by, or associate with albumin fusion proteins of the present invention in a sample derived from a host are well-known to those of skill in the art. Such assay methods include radioimmunoassays, competitive-binding assays, Western Blot analysis and ELISA assays. Assaying polypeptide levels in a biological sample can occur using any art-known method.

Assaying polypeptide levels in a biological sample can occur using a variety of techniques. For example, polypeptide expression in tissues can be studied with classical immunohistological methods (Jalkanen et al., J. Cell. Biol. 101:976-985 (1985); Jalkanen, M., et al., J. Cell. Biol. 105:3087-3096 (1987)). Other methods useful for detecting polypeptide gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes, such as iodine ( $^{125}\text{I}$ ,  $^{121}\text{I}$ ), carbon ( $^{14}\text{C}$ ), sulfur ( $^{35}\text{S}$ ), tritium ( $^3\text{H}$ ), indium ( $^{112}\text{In}$ ), and technetium ( $^{99\text{m}}\text{Tc}$ ), and fluorescent labels, such as fluorescein and rhodamine, and biotin.

The tissue or cell type to be analyzed will generally include those which are known, or suspected, to express the gene of interest (such as, for example, cancer). The protein isolation methods employed herein may, for example, be such as those described

in Harlow and Lane (Harlow, E. and Lane, D., 1988, "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York), which is incorporated herein by reference in its entirety. The isolated cells can be derived from cell culture or from a patient. The analysis of cells taken from culture may be a necessary step  
5 in the assessment of cells that could be used as part of a cell-based gene therapy technique or, alternatively, to test the effect of compounds on the expression of the gene.

For example, albumin fusion proteins may be used to quantitatively or qualitatively detect the presence of polypeptides that bind to, are bound by, or associate with albumin fusion proteins of the present invention. This can be accomplished, for example, by  
10 immunofluorescence techniques employing a fluorescently labeled albumin fusion protein coupled with light microscopic, flow cytometric, or fluorimetric detection.

In a preferred embodiment, albumin fusion proteins comprising at least a fragment or variant of an antibody that specifically binds at least a Therapeutic protein disclosed herein (e.g., the Therapeutic proteins disclosed in Table 1) or otherwise known in the art  
15 may be used to quantitatively or qualitatively detect the presence of gene products or conserved variants or peptide fragments thereof. This can be accomplished, for example, by immunofluorescence techniques employing a fluorescently labeled antibody coupled with light microscopic, flow cytometric, or fluorimetric detection.

The albumin fusion proteins of the present invention may, additionally, be  
20 employed histologically, as in immunofluorescence, immunoelectron microscopy or non-immunological assays, for in situ detection of polypeptides that bind to, are bound by, or associate with an albumin fusion protein of the present invention. In situ detection may be accomplished by removing a histological specimen from a patient, and applying thereto a labeled antibody or polypeptide of the present invention. The albumin fusion proteins  
25 are preferably applied by overlaying the labeled albumin fusion proteins onto a biological sample. Through the use of such a procedure, it is possible to determine not only the presence of the polypeptides that bind to, are bound by, or associate with albumin fusion proteins, but also its distribution in the examined tissue. Using the present invention, those of ordinary skill will readily perceive that any of a wide variety of histological  
30 methods (such as staining procedures) can be modified in order to achieve such in situ detection.

Immunoassays and non-immunoassays that detect polypeptides that bind to, are

bound by, or associate with albumin fusion proteins will typically comprise incubating a sample, such as a biological fluid, a tissue extract, freshly harvested cells, or lysates of cells which have been incubated in cell culture, in the presence of a detectably labeled antibody capable of binding gene products or conserved variants or peptide fragments thereof, and detecting the bound antibody by any of a number of techniques well-known in the art.

The biological sample may be brought in contact with and immobilized onto a solid phase support or carrier such as nitrocellulose, or other solid support which is capable of immobilizing cells, cell particles or soluble proteins. The support may then be washed with suitable buffers followed by treatment with the detectably labeled albumin fusion protein of the invention. The solid phase support may then be washed with the buffer a second time to remove unbound antibody or polypeptide. Optionally the antibody is subsequently labeled. The amount of bound label on solid support may then be detected by conventional means.

By "solid phase support or carrier" is intended any support capable of binding a polypeptide (e.g., an albumin fusion protein, or polypeptide that binds, is bound by, or associates with an albumin fusion protein of the invention.) Well-known supports or carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, gabbros, and magnetite. The nature of the carrier can be either soluble to some extent or insoluble for the purposes of the present invention. The support material may have virtually any possible structural configuration so long as the coupled molecule is capable of binding to a polypeptide. Thus, the support configuration may be spherical, as in a bead, or cylindrical, as in the inside surface of a test tube, or the external surface of a rod. Alternatively, the surface may be flat such as a sheet, test strip, etc. Preferred supports include polystyrene beads. Those skilled in the art will know many other suitable carriers for binding antibody or antigen, or will be able to ascertain the same by use of routine experimentation.

The binding activity of a given lot of albumin fusion protein may be determined according to well known methods. Those skilled in the art will be able to determine operative and optimal assay conditions for each determination by employing routine experimentation.

In addition to assaying polypeptide levels in a biological sample obtained from an

individual, polypeptide can also be detected *in vivo* by imaging. For example, in one embodiment of the invention, albumin fusion proteins of the invention are used to image diseased or neoplastic cells.

Labels or markers for *in vivo* imaging of albumin fusion proteins of the invention include those detectable by X-radiography, NMR, MRI, CAT-scans or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which may be incorporated into the albumin fusion protein by labeling of nutrients of a cell line (or bacterial or yeast strain) engineered.

Additionally, albumin fusion proteins of the invention whose presence can be detected, can be administered. For example, albumin fusion proteins of the invention labeled with a radio-opaque or other appropriate compound can be administered and visualized *in vivo*, as discussed, above for labeled antibodies. Further, such polypeptides can be utilized for *in vitro* diagnostic procedures.

A polypeptide-specific antibody or antibody fragment which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (for example,  $^{131}\text{I}$ ,  $^{112}\text{In}$ ,  $^{99\text{m}}\text{Tc}$ ), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (for example, parenterally, subcutaneously or intraperitoneally) into the mammal to be examined for a disorder. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of  $^{99\text{m}}\text{Tc}$ . The labeled albumin fusion protein will then preferentially accumulate at the locations in the body which contain a polypeptide or other substance that binds to, is bound by or associates with an albumin fusion protein of the present invention. *In vivo* tumor imaging is described in S.W. Burchiel et al., "Immunopharmacokinetics of Radiolabeled Antibodies and Their Fragments" (Chapter 13 in *Tumor Imaging: The Radiochemical Detection of Cancer*, S.W. Burchiel and B. A. Rhodes, eds., Masson Publishing Inc. (1982)).

One of the ways in which an albumin fusion protein of the present invention can be detectably labeled is by linking the same to a reporter enzyme and using the linked product

in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)", 1978, Diagnostic Horizons 2:1-7, Microbiological Associates Quarterly Publication, Walkersville, MD); Voller et al., *J. Clin. Pathol.* 31:507-520 (1978); Butler, J.E., *Meth. Enzymol.* 73:482-523 (1981); Maggio, E. (ed.), 1980, Enzyme Immunoassay, CRC Press, Boca Raton, FL.; Ishikawa, E. et al., (eds.), 1981, Enzyme Immunoassay, 5 Kgaku Shoin, Tokyo). The reporter enzyme which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Reporter enzymes which can be used to detectably label 10 the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the 15 detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the reporter enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

Albumin fusion proteins may also be radiolabelled and used in any of a variety of 20 other immunoassays. For example, by radioactively labeling the albumin fusion proteins, it is possible to use the albumin fusion proteins in a radioimmunoassay (RIA) (see, for example, Weintraub, B., Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by means 25 including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

It is also possible to label the albumin fusion proteins with a fluorescent compound. When the fluorescently labeled antibody is exposed to light of the proper wave length, its presence can then be detected due to fluorescence. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, 30 rhodamine, phycoerythrin, phycocyanin, allophycocyanin, ophthaldehyde and fluorescamine.

The albumin fusion protein can also be detectably labeled using fluorescence

emitting metals such as  $^{152}\text{Eu}$ , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

The albumin fusion proteins can also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged albumin fusion protein is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

Likewise, a bioluminescent compound may be used to label albumin fusion proteins of the present invention. Bioluminescence is a type of chemiluminescence found in biological systems in, which a catalytic protein increases the efficiency of the chemiluminescent reaction. The presence of a bioluminescent protein is determined by detecting the presence of luminescence. Important bioluminescent compounds for purposes of labeling are luciferin, luciferase and aequorin.

### **Transgenic Organisms**

Transgenic organisms that express the albumin fusion proteins of the invention are also included in the invention. Transgenic organisms are genetically modified organisms into which recombinant, exogenous or cloned genetic material has been transferred. Such genetic material is often referred to as a transgene. The nucleic acid sequence of the transgene may include one or more transcriptional regulatory sequences and other nucleic acid sequences such as introns, that may be necessary for optimal expression and secretion of the encoded protein. The transgene may be designed to direct the expression of the encoded protein in a manner that facilitates its recovery from the organism or from a product produced by the organism, *e.g.* from the milk, blood, urine, eggs, hair or seeds of the organism. The transgene may consist of nucleic acid sequences derived from the genome of the same species or of a different species than the species of the target animal. The transgene may be integrated either at a locus of a genome where that particular nucleic acid sequence is not otherwise normally found or at the normal locus for the transgene.

The term "germ cell line transgenic organism" refers to a transgenic organism in which the genetic alteration or genetic information was introduced into a germ line cell,

thereby conferring the ability of the transgenic organism to transfer the genetic information to offspring. If such offspring in fact possess some or all of that alteration or genetic information, then they too are transgenic organisms. The alteration or genetic information may be foreign to the species of organism to which the recipient belongs, foreign only to the particular individual recipient, or may be genetic information already possessed by the recipient. In the last case, the altered or introduced gene may be expressed differently than the native gene.

A transgenic organism may be a transgenic animal or a transgenic plant. Transgenic animals can be produced by a variety of different methods including transfection, electroporation, microinjection, gene targeting in embryonic stem cells and recombinant viral and retroviral infection (*see, e.g.*, U.S. Patent No. 4,736,866; U.S. Patent No. 5,602,307; Mullins *et al.* (1993) *Hypertension* 22(4):630-633; Brenin *et al.* (1997) *Surg. Oncol.* 6(2):99-110; Tuan (ed.), *Recombinant Gene Expression Protocols*, Methods in Molecular Biology No. 62, Humana Press (1997)). The method of introduction of nucleic acid fragments into recombination competent mammalian cells can be by any method which favors co-transformation of multiple nucleic acid molecules. Detailed procedures for producing transgenic animals are readily available to one skilled in the art, including the disclosures in U.S. Patent No. 5,489,743 and U.S. Patent No. 5,602,307.

A number of recombinant or transgenic mice have been produced, including those which express an activated oncogene sequence (U.S. Patent No. 4,736,866); express simian SV40 T-antigen (U.S. Patent No. 5,728,915); lack the expression of interferon regulatory factor 1 (IRF-1) (U.S. Patent No. 5,731,490); exhibit dopaminergic dysfunction (U.S. Patent No. 5,723,719); express at least one human gene which participates in blood pressure control (U.S. Patent No. 5,731,489); display greater similarity to the conditions existing in naturally occurring Alzheimer's disease (U.S. Patent No. 5,720,936); have a reduced capacity to mediate cellular adhesion (U.S. Patent No. 5,602,307); possess a bovine growth hormone gene (Clutter *et al.* (1996) *Genetics* 143(4):1753-1760); or, are capable of generating a fully human antibody response (McCarthy (1997) *The Lancet* 349(9049):405).

While mice and rats remain the animals of choice for most transgenic experimentation, in some instances it is preferable or even necessary to use alternative

animal species. Transgenic procedures have been successfully utilized in a variety of non-murine animals, including sheep, goats, pigs, dogs, cats, monkeys, chimpanzees, hamsters, rabbits, cows and guinea pigs (see, e.g., Kim *et al.* (1997) *Mol. Reprod. Dev.* 46(4):515-526; Houdebine (1995) *Reprod. Nutr. Dev.* 35(6):609-617; Petters (1994) *Reprod. Fertil. Dev.* 6(5):643-645; Schnieke *et al.* (1997) *Science* 278(5346):2130-2133; and Amoah (1997) *J. Animal Science* 75(2):578-585).

To direct the secretion of the transgene-encoded protein of the invention into the milk of transgenic mammals, it may be put under the control of a promoter that is preferentially activated in mammary epithelial cells. Promoters that control the genes encoding milk proteins are preferred, for example the promoter for casein, beta lactoglobulin, whey acid protein, or lactalbumin (see, e.g., DiTullio (1992) *BioTechnology* 10:74-77; Clark *et al.* (1989) *BioTechnology* 7:487-492; Gorton *et al.* (1987) *BioTechnology* 5:1183-1187; and Soulier *et al.* (1992) *FEBS Letts.* 297:13). The transgenic mammals of choice would produce large volumes of milk and have long lactating periods, for example goats, cows, camels or sheep.

An albumin fusion protein of the invention can also be expressed in a transgenic plant, e.g. a plant in which the DNA transgene is inserted into the nuclear or plastidic genome. Plant transformation procedures used to introduce foreign nucleic acids into plant cells or protoplasts are known in the art (e.g., see Example 19). See, in general, Methods in Enzymology Vol. 153 ("Recombinant DNA Part D") 1987, Wu and Grossman Eds., Academic Press and European Patent Application EP 693554. Methods for generation of genetically engineered plants are further described in US Patent No. 5,283,184, US Patent No. 5, 482,852, and European Patent Application EP 693 554, all of which are hereby incorporated by reference.

#### **Pharmaceutical or Therapeutic Compositions**

The albumin fusion proteins of the invention or formulations thereof may be administered by any conventional method including parenteral (e.g. subcutaneous or intramuscular) injection or intravenous infusion. The treatment may consist of a single dose or a plurality of doses over a period of time.

While it is possible for an albumin fusion protein of the invention to be administered alone, it is preferable to present it as a pharmaceutical formulation, together



with one or more acceptable carriers. The carrier(s) must be "acceptable" in the sense of being compatible with the albumin fusion protein and not deleterious to the recipients thereof. Typically, the carriers will be water or saline which will be sterile and pyrogen free. Albumin fusion proteins of the invention are particularly well suited to formulation in aqueous carriers such as sterile pyrogen free water, saline or other isotonic solutions because of their extended shelf-life in solution. For instance, pharmaceutical compositions of the invention may be formulated well in advance in aqueous form, for instance, weeks or months or longer time periods before being dispensed.

For example, wherein the Therapeutic protein is hGH, EPO, alpha-IFN or beta-IFN, formulations containing the albumin fusion protein may be prepared taking into account the extended shelf-life of the albumin fusion protein in aqueous formulations. As exhibited in Table 2, most Therapeutic proteins are unstable with short shelf-lives after formulation with an aqueous carrier. As discussed above, the shelf-life of many of these Therapeutic proteins are markedly increased or prolonged after fusion to HA.

**Table 2**

Protein	Tradename, Manufacturer	Route	Formulation	Storage Conditions of Non-Fusion Protein
Interferon, alpha-2a	Roferon-A, Hoffmann- LaRoche	sc im	sol n (vial or pre-filled syringe)	4-8°C
Interferon, alpha-2b	Intron-A, Schering Plough	iv sc im	sol n; powder + dil.	4-8°C (all preps, before and after dilution)
COMBO Interferon alpha-2b + Ribavirin	Rebetron (Intron-A + Rebetol) Schering Plough	po + sc	Rebetol capsule + Intron-A injection	
Interferon,	Infergen	sc	sol n	4-8°C

Protein	Tradename, Manufacturer	Route	Formulation	Storage Conditions of Non-Fusion Protein
Alphacon-1	Amgen			
Interferon, alpha-n1, Lympho- blastoid	Wellferon, Wellcome	sc im	sol n (with albumin as stablizer )	4-8°C
Interferon, beta-1a	Avonex, Biogen	im	powder + dil. (with albumin)	4-8°C (before and after dilution) (Use within 3-6h of reconstitution)
	Rebif, Ares-Serono (Europe only)	sc	sol n, in pre-filled syringe	
Interferon, beta-1b	Betaseron, Chiron (Europe: Betaferon)	sc	powder + dil. (with albumin)	4-8°C (before and after dilution) (Use within 3h of reconstitution) Single use vials.
Interferon, Gamma-1b	Actimmune, InterMune Pharmaceuticals	sc		4-8°C (before and after dilution) (Use within 3h of reconstitution).
Growth	Genotropin,		powder/dil	4-8°C

Protein	Tradename, Manufacturer	Route	Formulation	Storage Conditions of Non-Fusion Protein
Hormone (somatropin)	Pharmacia Upjohn		cartridges (single or multi-use); single use MiniQuick injector	(before and after dilution); single use MiniQuick Delivery Device should be refrigerated until use.
	Humatrope, Eli Lilly	sc im	powder + dil. (Vial or pen cartridge)	4-8°C (before and after dilution) (Use vials within 25h, cartridges within 28d, of reconstitution).
	Norditropin, Novo Nordisk Pharmaceuticals			
	Nutropin, Genentech	sc	powder + dil.	4-8°C (stable for 14d after dilution) (all preps, before and after dilution)
	Nutropin AQ, Genentech	sc	solution	4-8°C (Stable for 28 d after 1st use)
	Nutropin Depot, Genentech	sc	microsphere suspension as powder + dil.	4-8°C Single use pkges. Dose 1-2x/month

Protein	Tradename, Manufacturer	Route	Formulation	Storage Conditions of Non-Fusion Protein
				(ProLease micro- encapsulation technol.)
	Saizen, (Serono)	sc im	powder + dil.	Powder should be stored at Rm Temp . After reconstitution store 4-8°C for up to 14d.
	Serostim, Serono			Powder should be stored at Rm Temp . After reconstitution store in 4-8°C for up to 14d.
hGH, with N-term. Met (somatrem)	Protropin, Genentech	sc im	powder + dil.	4-8°C (all preps, before and after dilution)
Erythropoieti n (Epoetin alfa)	Epogen, Amgen	iv sc	sol n	4-8°C (use within 21d of first use) (Single & multi-dose vials)
	Procrit, Amgen	iv sc	sol n	4-8°C (use within 21d of first use) (Single & multi-dose

Protein	Tradename, Manufacturer	Route	Formulation	Storage Conditions of Non-Fusion Protein vials)

In instances where aerosol administration is appropriate, the albumin fusion proteins of the invention can be formulated as aerosols using standard procedures. The term "aerosol" includes any gas-borne suspended phase of an albumin fusion protein of the instant invention which is capable of being inhaled into the bronchioles or nasal passages. Specifically, aerosol includes a gas-borne suspension of droplets of an albumin fusion protein of the instant invention, as may be produced in a metered dose inhaler or nebulizer, or in a mist sprayer. Aerosol also includes a dry powder composition of a compound of the instant invention suspended in air or other carrier gas, which may be delivered by insufflation from an inhaler device, for example. See Ganderton & Jones, *Drug Delivery to the Respiratory Tract*, Ellis Horwood (19 87); Gonda (1990) *Critical Reviews in Therapeutic Drug Carrier Systems* 6:273-313; and Raeburn *et al.*, (1992) *Pharmacol. Toxicol. Methods* 27:143-159.

The formulations of the invention are also typically non-immunogenic, in part, because of the use of the components of the albumin fusion protein being derived from the proper species. For instance, for human use, both the Therapeutic protein and albumin portions of the albumin fusion protein will typically be human. In some cases, wherein either component is non human-derived, that component may be humanized by substitution of key amino acids so that specific epitopes appear to the human immune system to be human in nature rather than foreign.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the albumin fusion protein with the carrier that constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation appropriate for the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampules, vials or syringes, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders. Dosage formulations may contain the Therapeutic protein portion at a lower molar concentration or lower dosage compared to the non-fused standard formulation for the Therapeutic protein given the extended serum half-life exhibited by many of the albumin fusion proteins of the invention.

As an example, when an albumin fusion protein of the invention comprises growth hormone as one or more of the Therapeutic protein regions, the dosage form can be calculated on the basis of the potency of the albumin fusion protein relative to the potency of hGH, while taking into account the prolonged serum half-life and shelf-life of the albumin fusion proteins compared to that of native hGH. Growth hormone is typically administered at 0.3 to 30.0 IU/kg/week, for example 0.9 to 12.0 IU/kg/week, given in three or seven divided doses for a year or more. In an albumin fusion protein consisting of full length HA fused to full length GH, an equivalent dose in terms of units would represent a greater weight of agent but the dosage frequency can be reduced, for example to twice a week, once a week or less.

Formulations or compositions of the invention may be packaged together with, or included in a kit with, instructions or a package insert referring to the extended shelf-life of the albumin fusion protein component. For instance, such instructions or package inserts may address recommended storage conditions, such as time, temperature and light, taking into account the extended or prolonged shelf-life of the albumin fusion proteins of the invention. Such instructions or package inserts may also address the particular advantages of the albumin fusion proteins of the inventions, such as the ease of storage for formulations that may require use in the field, outside of controlled hospital, clinic or office conditions. As described above, formulations of the invention may be in aqueous

form and may be stored under less than ideal circumstances without significant loss of therapeutic activity.

Albumin fusion proteins of the invention can also be included in nutraceuticals. For instance, certain albumin fusion proteins of the invention may be administered in natural products, including milk or milk product obtained from a transgenic mammal which expresses albumin fusion protein. Such compositions can also include plant or plant products obtained from a transgenic plant which expresses the albumin fusion protein. The albumin fusion protein can also be provided in powder or tablet form, with or without other known additives, carriers, fillers and diluents. Nutraceuticals are described in Scott Hegenhart, *Food Product Design*, Dec. 1993.

The invention also provides methods of treatment and/or prevention of diseases or disorders (such as, for example, any one or more of the diseases or disorders disclosed herein) by administration to a subject of an effective amount of an albumin fusion protein of the invention or a polynucleotide encoding an albumin fusion protein of the invention ("albumin fusion polynucleotide") in a pharmaceutically acceptable carrier.

The albumin fusion protein and/or polynucleotide will be formulated and dosed in a fashion consistent with good medical practice, taking into account the clinical condition of the individual patient (especially the side effects of treatment with the albumin fusion protein and/or polynucleotide alone), the site of delivery, the method of administration, the scheduling of administration, and other factors known to practitioners. The "effective amount" for purposes herein is thus determined by such considerations.

As a general proposition, the total pharmaceutically effective amount of the albumin fusion protein administered parenterally per dose will be in the range of about 1 ug/kg/day to 10 mg/kg/day of patient body weight, although, as noted above, this will be subject to therapeutic discretion. More preferably, this dose is at least 0.01 mg/kg/day, and most preferably for humans between about 0.01 and 1 mg/kg/day for the hormone. If given continuously, the albumin fusion protein is typically administered at a dose rate of about 1 ug/kg/hour to about 50 ug/kg/hour, either by 1-4 injections per day or by continuous subcutaneous infusions, for example, using a mini-pump. An intravenous bag solution may also be employed. The length of treatment needed to observe changes and the interval following treatment for responses to occur appears to vary depending on the desired effect.

Albumin fusion proteins and/or polynucleotides can be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion.

Albumin fusion proteins and/or polynucleotides of the invention are also suitably administered by sustained-release systems. Examples of sustained-release albumin fusion proteins and/or polynucleotides are administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, gels, drops or transdermal patch), buccally, or as an oral or nasal spray. "Pharmaceutically acceptable carrier" refers to a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type. The term "parenteral" as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intraarticular injection and infusion. Additional examples of sustained-release albumin fusion proteins and/or polynucleotides include suitable polymeric materials (such as, for example, semi-permeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules), suitable hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, and sparingly soluble derivatives (such as, for example, a sparingly soluble salt).

Sustained-release matrices include polylactides (U.S. Pat. No. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma-ethyl-L-glutamate (Sidman et al., Biopolymers 22:547-556 (1983)), poly (2- hydroxyethyl methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167-277 (1981), and Langer, Chem. Tech. 12:98-105 (1982)), ethylene vinyl acetate (Langer et al., Id.) or poly-D- (-)-3-hydroxybutyric acid (EP 133,988).

Sustained-release albumin fusion proteins and/or polynucleotides also include liposomally entrapped albumin fusion proteins and/or polynucleotides of the invention (see generally, Langer, *Science* 249:1527-1533 (1990); Treat et al., in *Liposomes in the Therapy of Infectious Disease and Cancer*, Lopez-Berestein and Fidler (eds.), Liss, New York, pp. 317 -327 and 353-365 (1989)). Liposomes containing the albumin fusion protein



and/or polynucleotide are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. (USA) 82:3688-3692 (1985); Hwang et al., Proc. Natl. Acad. Sci.(USA) 77:4030-4034 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese Pat. Appl. 83-118008; U.S. Pat. Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily, the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mol. percent cholesterol, the selected proportion being adjusted for the optimal Therapeutic.

In yet an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are delivered by way of a pump (*see* Langer, *supra*; Sefton, CRC Crit. Ref. Biomed. Eng. 14:201 (1987); Buchwald et al., Surgery 88:507 (1980); Saudek et al., N. Engl. J. Med. 321:574 (1989)).

Other controlled release systems are discussed in the review by Langer (*Science* 249:1527-1533 (1990)).

For parenteral administration, in one embodiment, the albumin fusion protein and/or polynucleotide is formulated generally by mixing it at the desired degree of purity, in a unit dosage injectable form (solution, suspension, or emulsion), with a pharmaceutically acceptable carrier, i.e., one that is non-toxic to recipients at the dosages and concentrations employed and is compatible with other ingredients of the formulation. For example, the formulation preferably does not include oxidizing agents and other compounds that are known to be deleterious to the Therapeutic.

Generally, the formulations are prepared by contacting the albumin fusion protein and/or polynucleotide uniformly and intimately with liquid carriers or finely divided solid carriers or both. Then, if necessary, the product is shaped into the desired formulation. Preferably the carrier is a parenteral carrier, more preferably a solution that is isotonic with the blood of the recipient. Examples of such carrier vehicles include water, saline, Ringer's solution, and dextrose solution. Non-aqueous vehicles such as fixed oils and ethyl oleate are also useful herein, as well as liposomes.

The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues) polypeptides, e.g., polyarginine

or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids, such as glycine, glutamic acid, aspartic acid, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, manose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counterions such as sodium; and/or nonionic surfactants such as polysorbates, poloxamers, or PEG.

The albumin fusion protein is typically formulated in such vehicles at a concentration of about 0.1 mg/ml to 100 mg/ml, preferably 1-10 mg/ml, at a pH of about 3 to 8. It will be understood that the use of certain of the foregoing excipients, carriers, or stabilizers will result in the formation of polypeptide salts.

Any pharmaceutical used for therapeutic administration can be sterile. Sterility is readily accomplished by filtration through sterile filtration membranes (e.g., 0.2 micron membranes). Albumin fusion proteins and/or polynucleotides generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

Albumin fusion proteins and/or polynucleotides ordinarily will be stored in unit or multi-dose containers, for example, sealed ampoules or vials, as an aqueous solution or as a lyophilized formulation for reconstitution. As an example of a lyophilized formulation, 10-ml vials are filled with 5 ml of sterile-filtered 1% (w/v) aqueous albumin fusion protein and/or polynucleotide solution, and the resulting mixture is lyophilized. The infusion solution is prepared by reconstituting the lyophilized albumin fusion protein and/or polynucleotide using bacteriostatic Water-for-Injection.

In a specific and preferred embodiment, the Albumin fusion protein formulations comprises 0.01 M sodium phosphate, 0.15 mM sodium chloride, 0.16 micromole sodium octanoate/milligram of fusion protein, 15 micrograms/milliliter polysorbate 80, pH 7.2. In another specific and preferred embodiment, the Albumin fusion protein formulations consists 0.01 M sodium phosphate, 0.15 mM sodium chloride, 0.16 micromole sodium octanoate/milligram of fusion protein, 15 micrograms/milliliter polysorbate 80, pH 7.2. The pH and buffer are chosen to match physiological conditions and the salt is added as a tonicifier. Sodium octanoate has been chosen due to its reported ability to increase the thermal stability of the protein in solution. Finally, polysorbate has been added as a generic surfactant, which lowers the surface tension of the solution and lowers non-

specific adsorption of the albumin fusion protein to the container closure system.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the albumin fusion proteins and/or polynucleotides of the invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition, the albumin fusion proteins and/or polynucleotides may be employed in conjunction with other therapeutic compounds.

The albumin fusion proteins and/or polynucleotides of the invention may be administered alone or in combination with adjuvants. Adjuvants that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, alum, alum plus deoxycholate (ImmunoAg), MTP-PE (Biocine Corp.), QS21 (Genentech, Inc.), BCG (e.g., THERACYS®), MPL and nonviable preparations of *Corynebacterium parvum*. In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with alum. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with QS-21. Further adjuvants that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Monophosphoryl lipid immunomodulator, AdjuVax 100a, QS-21, QS-18, CRL1005, Aluminum salts, MF-59, and Virosomal adjuvant technology. Vaccines that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, vaccines directed toward protection against MMR (measles, mumps, rubella), polio, varicella, tetanus/diphtheria, hepatitis A, hepatitis B, *Haemophilus influenzae* B, whooping cough, pneumonia, influenza, Lyme's Disease, rotavirus, cholera, yellow fever, Japanese encephalitis, poliomyelitis, rabies, typhoid fever, and pertussis. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the

compounds or agents given first, followed by the second.

The albumin fusion proteins and/or polynucleotides of the invention may be administered alone or in combination with other therapeutic agents. Albumin fusion protein and/or polynucleotide agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include but not limited to, chemotherapeutic agents, antibiotics, steroidal and non-steroidal anti-inflammatories, conventional immunotherapeutic agents, and/or therapeutic treatments described below. Combinations may be administered either concomitantly, e.g., as an admixture, separately but simultaneously or concurrently; or sequentially. This includes presentations in which the combined agents are administered together as a therapeutic mixture, and also procedures in which the combined agents are administered separately but simultaneously, e.g., as through separate intravenous lines into the same individual. Administration "in combination" further includes the separate administration of one of the compounds or agents given first, followed by the second.

In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an anticoagulant. Anticoagulants that may be administered with the compositions of the invention include, but are not limited to, heparin, low molecular weight heparin, warfarin sodium (e.g., COUMADIN®), dicumarol, 4-hydroxycoumarin, anisindione (e.g., MIRADON™), acenocoumarol (e.g., nicoumalone, SINTHROMET™), indan-1,3-dione, phenprocoumon (e.g., MARCUMAR™), ethyl biscoumacetate (e.g., TROMEXAN™), and aspirin. In a specific embodiment, compositions of the invention are administered in combination with heparin and/or warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin. In another specific embodiment, compositions of the invention are administered in combination with warfarin and aspirin. In another specific embodiment, compositions of the invention are administered in combination with heparin. In another specific embodiment, compositions of the invention are administered in combination with heparin and aspirin.

In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with thrombolytic drugs. Thrombolytic drugs that may be administered with the compositions of the invention include, but are not limited to, plasminogen, lys-plasminogen, alpha2-antiplasmin, streptokinae (e.g.,

KABIKINASE™), antirespace (e.g., EMINASE™), tissue plasminogen activator (t-PA, altevase, ACTIVASE™), urokinase (e.g., ABBOKINASE™), sauruplase, (Prourokinase, single chain urokinase), and aminocaproic acid (e.g., AMICAR™). In a specific embodiment, compositions of the invention are administered in combination with tissue plasminogen activator and aspirin.

In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with antiplatelet drugs. Antiplatelet drugs that may be administered with the compositions of the invention include, but are not limited to, aspirin, dipyridamole (e.g., PERSANTINE™), and ticlopidine (e.g., TICLID™).

In specific embodiments, the use of anti-coagulants, thrombolytic and/or antiplatelet drugs in combination with albumin fusion proteins and/or polynucleotides of the invention is contemplated for the prevention, diagnosis, and/or treatment of thrombosis, arterial thrombosis, venous thrombosis, thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the use of anticoagulants, thrombolytic drugs and/or antiplatelet drugs in combination with albumin fusion proteins and/or polynucleotides of the invention is contemplated for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the therapeutics of the invention, alone or in combination with antiplatelet, anticoagulant, and/or thrombolytic drugs, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

In certain embodiments, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with antiretroviral agents, nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and/or protease inhibitors (PIs). NRTIs that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, RETROVIR™ (zidovudine/AZT), VIDEX™ (didanosine/ddI), HIVID™ (zalcitabine/ddC), ZERIT™ (stavudine/d4T), EPIVIR™

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(lamivudine/3TC), and COMBIVIR™ (zidovudine/lamivudine). NNRTIs that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, VIRAMUNE™ (nevirapine), RESCRIPTOR™ (delavirdine), and SUSTIVA™ (efavirenz). Protease inhibitors that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to, CRIXIVAN™ (indinavir), NORVIR™ (ritonavir), INVIRASE™ (saquinavir), and VIRACEPT™ (nelfinavir). In a specific embodiment, antiretroviral agents, nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, and/or protease inhibitors may be used in any combination with albumin fusion proteins and/or polynucleotides of the invention to treat AIDS and/or to prevent or treat HIV infection.

Additional NRTIs include LODENOSINE™ (F-ddA; an acid-stable adenosine NRTI; Triangle/Abbott); COVIRACIL™ (emtricitabine/FTC; structurally related to lamivudine (3TC) but with 3- to 10-fold greater activity *in vitro*; Triangle/Abbott); dOTC (BCH-10652, also structurally related to lamivudine but retains activity against a substantial proportion of lamivudine-resistant isolates; Biochem Pharma); Adefovir (refused approval for anti-HIV therapy by FDA; Gilead Sciences); PREVEON® (Adefovir Dipivoxil, the active prodrug of adefovir; its active form is PMEA-pp); TENOFOVIR™ (bis-POC PMPA, a PMPA prodrug; Gilead); DAPD/DXG (active metabolite of DAPD; Triangle/Abbott); D-D4FC (related to 3TC, with activity against AZT/3TC-resistant virus); GW420867X (Glaxo Wellcome); ZIAGEN™ (abacavir/159U89; Glaxo Wellcome Inc.); CS-87 (3'-azido-2',3'-dideoxyuridine; WO 99/66936); and S-acyl-2-thioethyl (SATE)-bearing prodrug forms of  $\beta$ -L-FD4C and  $\beta$ -L-FddC (WO 98/17281).

Additional NNRTIs include COACTINON™ (Emivirine/MKC-442, potent NNRTI of the HEPT class; Triangle/Abbott); CAPRAVIRINE™ (AG-1549/S-1153, a next generation NNRTI with activity against viruses containing the K103N mutation; Agouron); PNU-142721 (has 20- to 50-fold greater activity than its predecessor delavirdine and is active against K103N mutants; Pharmacia & Upjohn); DPC-961 and DPC-963 (second-generation derivatives of efavirenz, designed to be active against viruses with the K103N mutation; DuPont); GW-420867X (has 25-fold greater activity than HBY097 and is active against K103N mutants; Glaxo Wellcome); CALANOLIDE A

(naturally occurring agent from the latex tree; active against viruses containing either or both the Y181C and K103N mutations); and Propolis (WO 99/49830).

Additional protease inhibitors include LOPINAVIR™ (ABT378/r; Abbott Laboratories); BMS-232632 (an azapeptide; Bristol-Myers Squibb); TIPRANAVIR™ (PNU-140690, a non-peptic dihydropyrone; Pharmacia & Upjohn); PD-178390 (a nonpeptidic dihydropyrone; Parke-Davis); BMS 232632 (an azapeptide; Bristol-Myers Squibb); L-756,423 (an indinavir analog; Merck); DMP-450 (a cyclic urea compound; Avid & DuPont); AG-1776 (a peptidomimetic with *in vitro* activity against protease inhibitor-resistant viruses; Agouron); VX-175/GW-433908 (phosphate prodrug of amprenavir; Vertex & Glaxo Wellcome); CGP61755 (Ciba); and AGENERASE™ (amprenavir; Glaxo Wellcome Inc.).

Additional antiretroviral agents include fusion inhibitors/gp41 binders. Fusion inhibitors/gp41 binders include T-20 (a peptide from residues 643-678 of the HIV gp41 transmembrane protein ectodomain which binds to gp41 in its resting state and prevents transformation to the fusogenic state; Trimeris) and T-1249 (a second-generation fusion inhibitor; Trimeris).

Additional antiretroviral agents include fusion inhibitors/chemokine receptor antagonists. Fusion inhibitors/chemokine receptor antagonists include CXCR4 antagonists such as AMD 3100 (a bicyclam), SDF-1 and its analogs, and ALX40-4C (a cationic peptide), T22 (an 18 amino acid peptide; Trimeris) and the T22 analogs T134 and T140; CCR5 antagonists such as RANTES (9-68), AOP-RANTES, NNY-RANTES, and TAK-779; and CCR5/CXCR4 antagonists such as NSC 651016 (a distamycin analog). Also included are CCR2B, CCR3, and CCR6 antagonists. Chemokine receptor agonists such as RANTES, SDF-1, MIP-1 $\alpha$ , MIP-1 $\beta$ , etc., may also inhibit fusion.

Additional antiretroviral agents include integrase inhibitors. Integrase inhibitors include dicaffeoylquinic (DFQA) acids; L-chicoric acid (a dicaffeoyltartaric (DCTA) acid); quinalizarin (QLC) and related anthraquinones; ZINTEVIR™ (AR 177, an oligonucleotide that probably acts at cell surface rather than being a true integrase inhibitor; Arondex); and naphthols such as those disclosed in WO 98/50347.

Additional antiretroviral agents include hydroxyurea-like compounds such as BCX-34 (a purine nucleoside phosphorylase inhibitor; Biocryst); ribonucleotide reductase inhibitors such as DIDOX™ (Molecules for Health); inosine monophosphate

dehydrogenase (IMPDH) inhibitors such as VX-497 (Vertex); and mycopholic acids such as CellCept (mycophenolate mofetil; Roche).

Additional antiretroviral agents include inhibitors of viral integrase, inhibitors of viral genome nuclear translocation such as arylene bis(methylketone) compounds; inhibitors of HIV entry such as AOP-RANTES, NNY-RANTES, RANTES-IgG fusion protein, soluble complexes of RANTES and glycosaminoglycans (GAG), and AMD-3100; nucleocapsid zinc finger inhibitors such as dithiane compounds; targets of HIV Tat and Rev; and pharmacoenhancers such as ABT-378.

Other antiretroviral therapies and adjunct therapies include cytokines and lymphokines such as MIP-1 $\alpha$ , MIP-1 $\beta$ , SDF-1 $\alpha$ , IL-2, PROLEUKIN™ (aldesleukin/L2-7001; Chiron), IL-4, IL-10, IL-12, and IL-13; interferons such as IFN- $\alpha$ 2a; antagonists of TNFs, NF $\kappa$ B, GM-CSF, M-CSF, and IL-10; agents that modulate immune activation such as cyclosporin and prednisone; vaccines such as Remune™ (HIV Immunogen), APL 400-003 (Apollon), recombinant gp120 and fragments, bivalent (B/E) recombinant envelope glycoprotein, rgp120CM235, MN rgp120, SF-2 rgp120, gp120/soluble CD4 complex, Delta JR-FL protein, branched synthetic peptide derived from discontinuous gp120 C3/C4 domain, fusion-competent immunogens, and Gag, Pol, Nef, and Tat vaccines; gene-based therapies such as genetic suppressor elements (GSEs; WO 98/54366), and intrakines (genetically modified CC chemokines targetted to the ER to block surface expression of newly synthesized CCR5 (Yang *et al.*, *PNAS* 94:11567-72 (1997); Chen *et al.*, *Nat. Med.* 3:1110-16 (1997)); antibodies such as the anti-CXCR4 antibody 12G5, the anti-CCR5 antibodies 2D7, 5C7, PA8, PA9, PA10, PA11, PA12, and PA14, the anti-CD4 antibodies Q4120 and RPA-T4, the anti-CCR3 antibody 7B11, the anti-gp120 antibodies 17b, 48d, 447-52D, 257-D, 268-D and 50.1, anti-Tat antibodies, anti-TNF- $\alpha$  antibodies, and monoclonal antibody 33A; aryl hydrocarbon (AH) receptor agonists and antagonists such as TCDD, 3,3',4,4',5-pentachlorobiphenyl, 3,3',4,4'-tetrachlorobiphenyl, and  $\alpha$ -naphthoflavone (WO 98/30213); and antioxidants such as  $\gamma$ -L-glutamyl-L-cysteine ethyl ester ( $\gamma$ -GCE; WO 99/56764).

In a further embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antiviral agent. Antiviral agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, acyclovir, ribavirin, amantadine, and remantidine.



In other embodiments, albumin fusion proteins and/or polynucleotides of the invention may be administered in combination with anti-opportunistic infection agents. Anti-opportunistic agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention, include, but are not limited to,

5 TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, ATOVAQUONE™, ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, ETHAMBUTOL™, RIFABUTIN™, CLARITHROMYCIN™, AZITHROMYCIN™, GANCICLOVIR™, FOSCARNET™, CIDOFOVIR™, FLUCONAZOLE™, ITRACONAZOLE™, KETOCONAZOLE™, ACYCLOVIR™, FAMCICOLVIR™,

10 PYRIMETHAMINE™, LEUCOVORIN™, NEUPOGEN™ (filgrastim/G-CSF), and LEUKINE™ (sargramostim/GM-CSF). In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with TRIMETHOPRIM-SULFAMETHOXAZOLE™, DAPSONE™, PENTAMIDINE™, and/or ATOVAQUONE™ to prophylactically treat or prevent an opportunistic *Pneumocystis*

15 *carinii* pneumonia infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with ISONIAZID™, RIFAMPIN™, PYRAZINAMIDE™, and/or ETHAMBUTOL™ to prophylactically treat or prevent an opportunistic *Mycobacterium avium* complex infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in

20 any combination with RIFABUTIN™, CLARITHROMYCIN™, and/or AZITHROMYCIN™ to prophylactically treat or prevent an opportunistic *Mycobacterium tuberculosis* infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with GANCICLOVIR™, FOSCARNET™, and/or CIDOFOVIR™ to prophylactically treat or prevent an

25 opportunistic cytomegalovirus infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with FLUCONAZOLE™, ITRACONAZOLE™, and/or KETOCONAZOLE™ to prophylactically treat or prevent an opportunistic fungal infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in

30 any combination with ACYCLOVIR™ and/or FAMCICOLVIR™ to prophylactically treat or prevent an opportunistic herpes simplex virus type I and/or type II infection. In another

specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with PYRIMETHAMINE™ and/or LEUCOVORIN™ to prophylactically treat or prevent an opportunistic *Toxoplasma gondii* infection. In another specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are used in any combination with LEUCOVORIN™ and/or NEUPOGEN™ to prophylactically treat or prevent an opportunistic bacterial infection.

In a further embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antibiotic agent. Antibiotic agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, amoxicillin, beta-lactamases, aminoglycosides, beta-lactam (glycopeptide), beta-lactamases, Clindamycin, chloramphenicol, cephalosporins, ciprofloxacin, erythromycin, fluoroquinolones, macrolides, metronidazole, penicillins, quinolones, rapamycin, rifampin, streptomycin, sulfonamide, tetracyclines, trimethoprim, trimethoprim-sulfamethoxazole, and vancomycin.

In other embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with immunestimulants. Immunestimulants that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, levamisole (e.g., ERGAMISOL™), isoprinosine (e.g. INOSIPLEX™), interferons (e.g. interferon alpha), and interleukins (e.g., IL-2).

In other embodiments, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with immunosuppressive agents. Immunosuppressive agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, steroids, cyclosporine, cyclosporine analogs, cyclophosphamide methylprednisone, prednisone, azathioprine, FK-506, 15-deoxyspergualin, and other immunosuppressive agents that act by suppressing the function of responding T cells. Other immunosuppressive agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, prednisolone, methotrexate, thalidomide, methoxsalen, rapamycin, leflunomide, mizoribine (BREDININ™), brequinar, deoxyspergualin, and azaspirane (SKF 105685), ORTHOCLONE OKT® 3 (muromonab-CD3), SANDIMMUNE™, NEORAL™,

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SANGDYA™ (cyclosporine), PROGRAF® (FK506, tacrolimus), CELLCEPT® (mycophenolate mofetil, of which the active metabolite is mycophenolic acid), IMURAN™ (azathioprine), glucocorticosteroids, adrenocortical steroids such as DELTASONE™ (prednisone) and HYDELTRASOL™ (prednisolone), FOLEX™ and  
5 MEXATE™ (methotrexate), OXSORALEN-ULTRA™ (methoxsalen) and RAPAMUNE™ (sirolimus). In a specific embodiment, immunosuppressants may be used to prevent rejection of organ or bone marrow transplantation.

In an additional embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered alone or in combination with one or more intravenous  
10 immune globulin preparations. Intravenous immune globulin preparations that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but not limited to, GAMMAR™, IVEEGAM™, SANDOGLOBULIN™, GAMMAGARD S/D™, ATGAM™ (antithymocyte globulin), and GAMIMUNE™. In a specific embodiment, albumin fusion proteins and/or polynucleotides of the invention are  
15 administered in combination with intravenous immune globulin preparations in transplantation therapy (e.g., bone marrow transplant).

In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered alone or in combination with an anti-inflammatory agent. Anti-inflammatory agents that may be administered with the albumin fusion proteins and/or  
20 polynucleotides of the invention include, but are not limited to, corticosteroids (e.g. betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, and triamcinolone), nonsteroidal anti-inflammatory drugs (e.g., diclofenac, diflunisal, etodolac, fenoprofen, floctafenine, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, oxaprozin, phenylbutazone, piroxicam, sulindac,  
25 tenoxicam, tiaprofenic acid, and tolmetin.), as well as antihistamines, aminoarylcarboxylic acid derivatives, arylacetic acid derivatives, arylbutyric acid derivatives, arylcarboxylic acids, arylpropionic acid derivatives, pyrazoles, pyrazolones, salicylic acid derivatives, thiazinecarboxamides, e-acetamidocaproic acid, S-adenosylmethionine, 3-amino-4-  
30 hydroxybutyric acid, amixetrine, bendazac, benzydamine, bucolome, difenpiramide, ditazol, emorfazone, guaiazulene, nabumetone, nimesulide, orgotein, oxaceprol, paranyline, perisoxal, pifoxime, proquazone, proxazole, and tenidap.

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In an additional embodiment, the compositions of the invention are administered alone or in combination with an anti-angiogenic agent. Anti-angiogenic agents that may be administered with the compositions of the invention include, but are not limited to, Angiostatin (Entremed, Rockville, MD), Troponin-1 (Boston Life Sciences, Boston, MA),  
5 anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel (Taxol), Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, VEGI, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum,  
10 tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes  
15 include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo  
20 complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate  
25 complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for  
30 example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include, but are not limited to,

platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, (1991)); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2-Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, (1992)); Cyclodextrin Tetradesulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, (1990)); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; (Takeuchi et al., Agents Actions 36:312-316, (1992)); and metalloproteinase inhibitors such as BB94.

Additional anti-angiogenic factors that may also be utilized within the context of the present invention include Thalidomide, (Celgene, Warren, NJ); Angiostatic steroid; AGM-1470 (H. Brem and J. Folkman *J Pediatr. Surg.* 28:445-51 (1993)); an integrin alpha v beta 3 antagonist (C. Storgard et al., *J Clin. Invest.* 103:47-54 (1999)); carboxynaminolmidazole; Carboxyamidotriazole (CAI) (National Cancer Institute, Bethesda, MD); Combretastatin A-4 (CA4P) (OXIGENE, Boston, MA); Squalamine (Magainin Pharmaceuticals, Plymouth Meeting, PA); TNP-470, (Tap Pharmaceuticals, Deerfield, IL); ZD-0101 AstraZeneca (London, UK); APRA (CT2584); Benefin, Byrostatin-1 (SC339555); CGP-41251 (PKC 412); CM101; Dexrazoxane (ICRF187); DMXAA; Endostatin; Flavopridiol; Genestein; GTE; ImmTher; Iressa (ZD1839); Octreotide (Somatostatin); Panretin; Penacillamine; Photopoint; PI-88; Prinomastat (AG-3340) Purytin; Suradista (FCE26644); Tamoxifen (Nolvadex); Tazarotene; Tetrathiomolybdate; Xeloda (Capecitabine); and 5-Fluorouracil.

Anti-angiogenic agents that may be administered in combination with the compounds of the invention may work through a variety of mechanisms including, but not limited to, inhibiting proteolysis of the extracellular matrix, blocking the function of endothelial cell-extracellular matrix adhesion molecules, by antagonizing the function of

angiogenesis inducers such as growth factors, and inhibiting integrin receptors expressed on proliferating endothelial cells. Examples of anti-angiogenic inhibitors that interfere with extracellular matrix proteolysis and which may be administered in combination with the compositions of the invention include, but are not limited to, AG-3340 (Agouron, La Jolla, CA), BAY-12-9566 (Bayer, West Haven, CT), BMS-275291 (Bristol Myers Squibb, Princeton, NJ), CGS-27032A (Novartis, East Hanover, NJ), Marimastat (British Biotech, Oxford, UK), and Metastat (Aeterna, St-Foy, Quebec). Examples of anti-angiogenic inhibitors that act by blocking the function of endothelial cell-extracellular matrix adhesion molecules and which may be administered in combination with the compositions of the invention include, but are not limited to, EMD-121974 (Merck KgaA Darmstadt, Germany) and Vitaxin (Ixsys, La Jolla, CA/Medimmune, Gaithersburg, MD). Examples of anti-angiogenic agents that act by directly antagonizing or inhibiting angiogenesis inducers and which may be administered in combination with the compositions of the invention include, but are not limited to, Angiozyme (Ribozyme, Boulder, CO), Anti-VEGF antibody (Genentech, S. San Francisco, CA), PTK-787/ZK-225846 (Novartis, Basel, Switzerland), SU-101 (Sugen, S. San Francisco, CA), SU-5416 (Sugen/ Pharmacia Upjohn, Bridgewater, NJ), and SU-6668 (Sugen). Other anti-angiogenic agents act to indirectly inhibit angiogenesis. Examples of indirect inhibitors of angiogenesis which may be administered in combination with the compositions of the invention include, but are not limited to, IM-862 (Cytran, Kirkland, WA), Interferon-alpha, IL-12 (Roche, Nutley, NJ), and Pentosan polysulfate (Georgetown University, Washington, DC).

In particular embodiments, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of an autoimmune disease, such as for example, an autoimmune disease described herein.

In a particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of arthritis. In a more particular embodiment, the use of compositions of the invention in combination with anti-angiogenic agents is contemplated for the treatment, prevention, and/or amelioration of rheumatoid arthritis.

In another embodiment, the polynucleotides encoding a polypeptide of the present invention are administered in combination with an angiogenic protein, or polynucleotides

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encoding an angiogenic protein. Examples of angiogenic proteins that may be administered with the compositions of the invention include, but are not limited to, acidic and basic fibroblast growth factors, VEGF-1, VEGF-2, VEGF-3, epidermal growth factor alpha and beta, platelet-derived endothelial cell growth factor, platelet-derived growth factor, tumor necrosis factor alpha, hepatocyte growth factor, insulin-like growth factor, colony stimulating factor, macrophage colony stimulating factor, granulocyte/macrophage colony stimulating factor, and nitric oxide synthase.

In additional embodiments, compositions of the invention are administered in combination with a chemotherapeutic agent. Chemotherapeutic agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to alkylating agents such as nitrogen mustards (for example, Mechlorethamine, cyclophosphamide, Cyclophosphamide Ifosfamide, Melphalan (L-sarcolysin), and Chlorambucil), ethylenimines and methylmelamines (for example, Hexamethylmelamine and Thiotepa), alkyl sulfonates (for example, Busulfan), nitrosoureas (for example, Carmustine (BCNU), Lomustine (CCNU), Semustine (methyl-CCNU), and Streptozocin (streptozotocin)), triazenes (for example, Dacarbazine (DTIC; dimethyltriazenoimidazolecarboxamide)), folic acid analogs (for example, Methotrexate (amethopterin)), pyrimidine analogs (for example, Fluorouacil (5-fluorouracil; 5-FU), Floxuridine (fluorodeoxyuridine; FudR), and Cytarabine (cytosine arabinoside)), purine analogs and related inhibitors (for example, Mercaptopurine (6-mercaptopurine; 6-MP), Thioguanine (6-thioguanine; TG), and Pentostatin (2'-deoxycoformycin)), vinca alkaloids (for example, Vinblastine (VLB, vinblastine sulfate)) and Vincristine (vincristine sulfate)), epipodophyllotoxins (for example, Etoposide and Teniposide), antibiotics (for example, Dactinomycin (actinomycin D), Daunorubicin (daunomycin; rubidomycin), Doxorubicin, Bleomycin, Plicamycin (mithramycin), and Mitomycin (mitomycin C), enzymes (for example, L-Asparaginase), biological response modifiers (for example, Interferon-alpha and interferon-alpha-2b), platinum coordination compounds (for example, Cisplatin (cis-DDP) and Carboplatin), anthracenedione (Mitoxantrone), substituted ureas (for example, Hydroxyurea), methylhydrazine derivatives (for example, Procarbazine (N-methylhydrazine; MIH), adrenocorticosteroids (for example, Prednisone), progestins (for example, Hydroxyprogesterone caproate, Medroxyprogesterone, Medroxyprogesterone acetate, and Megestrol acetate), estrogens (for example, Diethylstilbestrol (DES),

Diethylstilbestrol diphosphate, Estradiol, and Ethinyl estradiol), antiestrogens (for example, Tamoxifen), androgens (Testosterone propionate, and Fluoxymesterone), antiandrogens (for example, Flutamide), gonadotropin-releasing hormone analogs (for example, Leuprolide), other hormones and hormone analogs (for example, methyltestosterone, estramustine, estramustine phosphate sodium, chlorotrianisene, and testolactone), and others (for example, dicarbazine, glutamic acid, and mitotane).

In one embodiment, the compositions of the invention are administered in combination with one or more of the following drugs: infliximab (also known as Remicade™ Centocor, Inc.), Trocade (Roche, RO-32-3555), Leflunomide (also known as Arava™ from Hoechst Marion Roussel), Kineret™ (an IL-1 Receptor antagonist also known as Anakinra from Amgen, Inc.)

In a specific embodiment, compositions of the invention are administered in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or combination of one or more of the components of CHOP. In one embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies, human monoclonal anti-CD20 antibodies. In another embodiment, the compositions of the invention are administered in combination with anti-CD20 antibodies and CHOP, or anti-CD20 antibodies and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with Rituximab. In a further embodiment, compositions of the invention are administered with Rituximab and CHOP, or Rituximab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. In a specific embodiment, compositions of the invention are administered in combination with tositumomab. In a further embodiment, compositions of the invention are administered with tositumomab and CHOP, or tositumomab and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or prednisone. The anti-CD20 antibodies may optionally be associated with radioisotopes, toxins or cytotoxic prodrugs.

In another specific embodiment, the compositions of the invention are administered in combination Zevalin™. In a further embodiment, compositions of the invention are administered with Zevalin™ and CHOP, or Zevalin™ and any combination of one or more of the components of CHOP, particularly cyclophosphamide and/or



prednisone. Zevalin™ may be associated with one or more radisotopes. Particularly preferred isotopes are <sup>90</sup>Y and <sup>111</sup>In.

In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with cytokines. Cytokines that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, IL2, IL3, IL4, IL5, IL6, IL7, IL10, IL12, IL13, IL15, anti-CD40, CD40L, IFN-gamma and TNF-alpha. In another embodiment, albumin fusion proteins and/or polynucleotides of the invention may be administered with any interleukin, including, but not limited to, IL-1alpha, IL-1beta, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, IL-18, IL-19, IL-20, and IL-21.

In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with members of the TNF family. TNF, TNF-related or TNF-like molecules that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, soluble forms of TNF-alpha, lymphotoxin-alpha (LT-alpha, also known as TNF-beta), LT-beta (found in complex heterotrimer LT-alpha2-beta), OPGL, FasL, CD27L, CD30L, CD40L, 4-1BBL, DcR3, OX40L, TNF-gamma (International Publication No. WO 96/14328), AIM-I (International Publication No. WO 97/33899), endokine-alpha (International Publication No. WO 98/07880), OPG, and neutrokin-alpha (International Publication No. WO 98/18921, OX40, and nerve growth factor (NGF), and soluble forms of Fas, CD30, CD27, CD40 and 4-IBB, TR2 (International Publication No. WO 96/34095), DR3 (International Publication No. WO 97/33904), DR4 (International Publication No. WO 98/32856), TR5 (International Publication No. WO 98/30693), TRANK, TR9 (International Publication No. WO 98/56892), TR10 (International Publication No. WO 98/54202), 312C2 (International Publication No. WO 98/06842), and TR12, and soluble forms CD154, CD70, and CD153.

In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with angiogenic proteins. Angiogenic proteins that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Glioma Derived Growth Factor (GDGF), as disclosed in European Patent Number EP-399816; Platelet Derived Growth Factor-A

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(PDGF-A), as disclosed in European Patent Number EP-682110; Platelet Derived Growth Factor-B (PDGF-B), as disclosed in European Patent Number EP-282317; Placental Growth Factor (PlGF), as disclosed in International Publication Number WO 92/06194; Placental Growth Factor-2 (PlGF-2), as disclosed in Hauser et al., Growth Factors, 4:259-268 (1993); Vascular Endothelial Growth Factor (VEGF), as disclosed in International Publication Number WO 90/13649; Vascular Endothelial Growth Factor-A (VEGF-A), as disclosed in European Patent Number EP-506477; Vascular Endothelial Growth Factor-2 (VEGF-2), as disclosed in International Publication Number WO 96/39515; Vascular Endothelial Growth Factor B (VEGF-3); Vascular Endothelial Growth Factor B-186 (VEGF-B186), as disclosed in International Publication Number WO 96/26736; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/02543; Vascular Endothelial Growth Factor-D (VEGF-D), as disclosed in International Publication Number WO 98/07832; and Vascular Endothelial Growth Factor-E (VEGF-E), as disclosed in German Patent Number DE19639601. The above mentioned references are herein incorporated by reference in their entireties.

In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with Fibroblast Growth Factors. Fibroblast Growth Factors that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, and FGF-15.

In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with hematopoietic growth factors. Hematopoietic growth factors that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, granulocyte macrophage colony stimulating factor (GM-CSF) (sargramostim, LEUKINE™, PROKINE™), granulocyte colony stimulating factor (G-CSF) (filgrastim, NEUPOGEN™), macrophage colony stimulating factor (M-CSF, CSF-1) erythropoietin (epoetin alfa, EPOGEN™, PROCIT™), stem cell factor (SCF, c-kit ligand, steel factor), megakaryocyte colony stimulating factor, PIXY321 (a GMCSF/IL-3 fusion protein), interleukins, especially any one or more of IL-1 through IL-12, interferon-gamma, or thrombopoietin.

In certain embodiments, albumin fusion proteins and/or polynucleotides of the present invention are administered in combination with adrenergic blockers, such as, for example, acebutolol, atenolol, betaxolol, bisoprolol, carteolol, labetalol, metoprolol, nadolol, oxprenolol, penbutolol, pindolol, propranolol, sotalol, and timolol.

5 In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with an antiarrhythmic drug (e.g., adenosine, amidoarone, bretylium, digitalis, digoxin, digitoxin, diltiazem, disopyramide, esmolol, flecainide, lidocaine, mexiletine, moricizine, phenytoin, procainamide, N-acetyl procainamide, propafenone, propranolol, quinidine, sotalol, tocainide, and verapamil).

10 In another embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with diuretic agents, such as carbonic anhydrase-inhibiting agents (e.g., acetazolamide, dichlorophenamide, and methazolamide), osmotic diuretics (e.g., glycerin, isosorbide, mannitol, and urea), diuretics that inhibit  $\text{Na}^+\text{-K}^+\text{-2Cl}^-$  symport (e.g., furosemide, bumetanide, azosemide, piretanide, triparamide, 15 ethacrynic acid, muzolimine, and torsemide), thiazide and thiazide-like diuretics (e.g., bendroflumethiazide, benzthiazide, chlorothiazide, hydrochlorothiazide, hydroflumethiazide, methyclothiazide, polythiazide, trichormethiazide, chlorthalidone, indapamide, metolazone, and quinethazone), potassium sparing diuretics (e.g., amiloride and triamterene), and mineralcorticoid receptor antagonists (e.g., spironolactone, 20 canrenone, and potassium canrenoate).

In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for endocrine and/or hormone imbalance disorders. Treatments for endocrine and/or hormone imbalance disorders include, but are not limited to,  $^{127}\text{I}$ , radioactive isotopes of iodine such as  $^{131}\text{I}$  and  $^{123}\text{I}$ ; 25 recombinant growth hormone, such as HUMATROPE™ (recombinant somatropin); growth hormone analogs such as PROTROPIN™ (somatrem); dopamine agonists such as PARLODEL™ (bromocriptine); somatostatin analogs such as SANDOSTATIN™ (octreotide); gonadotropin preparations such as PREGNYL™, A.P.L.™ and PROFASI™ (chorionic gonadotropin (CG)), PERGONAL™ (menotropins), and METRODIN™ 30 (urofollitropin (uFSH)); synthetic human gonadotropin releasing hormone preparations such as FACTREL™ and LUTREPULSE™ (gonadorelin hydrochloride); synthetic gonadotropin agonists such as LUPRON™ (leuprolide acetate), SUPPRELIN™ (histrelin

acetate), SYNAREL™ (nafarelin acetate), and ZOLADEX™ (goserelin acetate); synthetic preparations of thyrotropin-releasing hormone such as RELEFACT TRH™ and THYPINONE™ (protirelin); recombinant human TSH such as THYROGEN™; synthetic preparations of the sodium salts of the natural isomers of thyroid hormones such as L-T<sub>4</sub>™, 5 SYNTHROID™ and LEVOTHROID™ (levothyroxine sodium), L-T<sub>3</sub>™, CYTOMEL™ and TRIOSTAT™ (liothyroine sodium), and THYROLAR™ (liotrix); antithyroid compounds such as 6-*n*-propylthiouracil (propylthiouracil), 1-methyl-2-mercaptoimidazole and TAPAZOLE™ (methimazole), NEO-MERCAZOLE™ (carbimazole); beta-adrenergic receptor antagonists such as propranolol and esmolol; Ca<sup>2+</sup> channel blockers; 10 dexamethasone and iodinated radiological contrast agents such as TELEPAQUE™ (iopanoic acid) and ORAGRAFIN™ (sodium ipodate).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, estrogens or conjugated estrogens such as ESTRACE™ (estradiol), ESTINYL™ (ethinyl estradiol), PREMARIN™, ESTRATAB™, ORTHO-EST™, OGEN™ 15 and estropipate (estrone), ESTROVIS™ (quinestrol), ESTRADERM™ (estradiol), DELESTROGEN™ and VALERGEN™ (estradiol valerate), DEPO-ESTRADIOL CYPIONATE™ and ESTROJECT LA™ (estradiol cypionate); antiestrogens such as NOLVADEX™ (tamoxifen), SEROPHENE™ and CLOMID™ (clomiphene); progestins such as DURALUTIN™ (hydroxyprogesterone caproate), MPA™ and DEPO- 20 PROVERA™ (medroxyprogesterone acetate), PROVERA™ and CYCRIN™ (MPA), MEGACE™ (megestrol acetate), NORLUTIN™ (norethindrone), and NORLUTATE™ and AYGESTIN™ (norethindrone acetate); progesterone implants such as NORPLANT SYSTEM™ (subdermal implants of norgestrel); antiprogestins such as RU 486™ (mifepristone); hormonal contraceptives such as ENOVID™ (norethynodrel plus 25 mestranol), PROGESTASERT™ (intrauterine device that releases progesterone), LOESTRIN™, BREVICON™, MODICON™, GENORA™, NELONA™, NORINYL™, OVACON-35™ and OVACON-50™ (ethinyl estradiol/norethindrone), LEVLEN™, NORDETTE™, TRI-LEVLEN™ and TRIPHASIL-21™ (ethinyl estradiol/levonorgestrel) LO/OVRAL™ and OVRAL™ (ethinyl estradiol/norgestrel), DEMULEN™ (ethinyl 30 estradiol/ethynodiol diacetate), NORINYL™, ORTHO-NOVUM™, NORETHIN™, GENORA™, and NELOVA™ (norethindrone/mestranol), DESOGEN™ and ORTHO-

CEPT™ (ethinyl estradiol/desogestrel), ORTHO-CYCLEN™ and ORTHO-TRICYCLEN™ (ethinyl estradiol/norgestimate), MICRONOR™ and NOR-QD™ (norethindrone), and OVRETTE™ (norgestrel).

Additional treatments for endocrine and/or hormone imbalance disorders include, but are not limited to, testosterone esters such as methenolone acetate and testosterone undecanoate; parenteral and oral androgens such as TESTOJECT-50™ (testosterone), TESTEX™ (testosterone propionate), DELATESTRYL™ (testosterone enanthate), DEPO-TESTOSTERONE™ (testosterone cypionate), DANOCRINE™ (danazol), HALOTESTIN™ (fluoxymesterone), ORETON METHYL™, TESTRED™ and VIRILON™ (methyltestosterone), and OXANDRIN™ (oxandrolone); testosterone transdermal systems such as TESTODERM™; androgen receptor antagonist and 5-alpha-reductase inhibitors such as ANDROCUR™ (cyproterone acetate), EULEXIN™ (flutamide), and PROSCAR™ (finasteride); adrenocorticotrophic hormone preparations such as CORTROSYN™ (cosyntropin); adrenocortical steroids and their synthetic analogs such as ACLOVATE™ (alclometasone dipropionate), CYCLOCORT™ (amcinonide), BECLOVENT™ and VANCERIL™ (beclomethasone dipropionate), CELESTONE™ (betamethasone), BENISONE™ and UTICORT™ (betamethasone benzoate), DIPROSONE™ (betamethasone dipropionate), CELESTONE PHOSPHATE™ (betamethasone sodium phosphate), CELESTONE SOLUSPAN™ (betamethasone sodium phosphate and acetate), BETA-VAL™ and VALISONE™ (betamethasone valerate), TEMOVATE™ (clobetasol propionate), CLODERM™ (clocortolone pivalate), CORTEF™ and HYDROCORTONE™ (cortisol (hydrocortisone)), HYDROCORTONE ACETATE™ (cortisol (hydrocortisone) acetate), LOCOID™ (cortisol (hydrocortisone) butyrate), HYDROCORTONE PHOSPHATE™ (cortisol (hydrocortisone) sodium phosphate), A-HYDROCORT™ and SOLU CORTEF™ (cortisol (hydrocortisone) sodium succinate), WESTCORT™ (cortisol (hydrocortisone) valerate), CORTISONE ACETATE™ (cortisone acetate), DESOWEN™ and TRIDESILON™ (desonide), TOPICORT™ (desoximetasone), DECADRON™ (dexamethasone), DECADRON LA™ (dexamethasone acetate), DECADRON PHOSPHATE™ and HEXADROL PHOSPHATE™ (dexamethasone sodium phosphate), FLORONE™ and MAXIFLOR™ (diflorasone diacetate), FLORINEF ACETATE™ (fludrocortisone acetate), AEROBID™ and

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NASALIDE™ (flunisolide), FLUONID™ and SYNALAR™ (fluocinolone acetonide), LIDEX™ (fluocinonide), FLUOR-OP™ and FML™ (fluorometholone), CORDRAN™ (flurandrenolide), HALOG™ (halcinonide), HMS LIZUIFILM™ (medrysone), MEDROL™ (methylprednisolone), DEPO-MEDROL™ and MEDROL ACETATE™ (methylprednisone acetate), A-METHAPRED™ and SOLUMEDROL™ (methylprednisolone sodium succinate), ELOCON™ (mometasone furoate), HALDRONE™ (paramethasone acetate), DELTA-CORTEF™ (prednisolone), ECONOPRED™ (prednisolone acetate), HYDELTRASOL™ (prednisolone sodium phosphate), HYDELTRA-T.B.A™ (prednisolone tebutate), DELTASON™ (prednisone), ARISTOCORT™ and KENACORT™ (triamcinolone), KENALOG™ (triamcinolone acetonide), ARISTOCORT™ and KENACORT DIACETATE™ (triamcinolone diacetate), and ARISTOSPAN™ (triamcinolone hexacetonide); inhibitors of biosynthesis and action of adrenocortical steroids such as CYTADREN™ (aminoglutethimide), NIZORAL™ (ketoconazole), MODRASTANE™ (trilostane), and METOPIRONE™ (metyrapone); bovine, porcine or human insulin or mixtures thereof; insulin analogs; recombinant human insulin such as HUMULIN™ and NOVOLIN™; oral hypoglycemic agents such as ORAMIDE™ and ORINASE™ (tolbutamide), DIABINESE™ (chlorpropamide), TOLAMIDE™ and TOLINASE™ (tolazamide), DYMELOR™ (acetoexamide), glibenclamide, MICRONASE™, DIBETA™ and GLYNASE™ (glyburide), GLUCOTROL™ (glipizide), and DIAMICRON™ (gliclazide), GLUCOPHAGE™ (metformin), ciglitazone, pioglitazone, and alpha-glucosidase inhibitors; bovine or porcine glucagon; somatostatins such as SANDOSTATIN™ (octreotide); and diazoxides such as PROGLYCEM™ (diazoxide).

In one embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for uterine motility disorders. Treatments for uterine motility disorders include, but are not limited to, estrogen drugs such as conjugated estrogens (e.g., PREMARIN® and ESTRATAB®), estradiols (e.g., CLIMARA® and ALORA®), estropipate, and chlorotrianisene; progestin drugs (e.g., AMEN® (medroxyprogesterone), MICRONOR® (norethidrone acetate), PROMETRIUM® progesterone, and megestrol acetate); and estrogen/progesterone combination therapies such as, for example, conjugated estrogens/medroxyprogesterone

(e.g., PREMPRO™ and PREMPHASE®) and norethindrone acetate/ethinyl estradiol (e.g., FEMHRT™).

In an additional embodiment, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with drugs effective in treating iron deficiency and hypochromic anemias, including but not limited to, ferrous sulfate (iron sulfate, FEOSOL™), ferrous fumarate (e.g., FEOSTAT™), ferrous gluconate (e.g., FERGON™), polysaccharide-iron complex (e.g., NIFEREX™), iron dextran injection (e.g., INFED™), cupric sulfate, pyroxidine, riboflavin, Vitamin B<sub>12</sub>, cyanocobalamin injection (e.g., REDISOL™, RUBRAMIN PC™), hydroxocobalamin, folic acid (e.g., FOLVITE™), leucovorin (folinic acid, 5-CHOH4PteGlu, citrovorum factor) or WELLCOVORIN (Calcium salt of leucovorin), transferrin or ferritin.

In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with agents used to treat psychiatric disorders. Psychiatric drugs that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, antipsychotic agents (e.g., chlorpromazine, chlorprothixene, clozapine, fluphenazine, haloperidol, loxapine, mesoridazine, molindone, olanzapine, perphenazine, pimozide, quetiapine, risperidone, thioridazine, thiothixene, trifluoperazine, and triflupromazine), antimanic agents (e.g., carbamazepine, divalproex sodium, lithium carbonate, and lithium citrate), antidepressants (e.g., amitriptyline, amoxapine, bupropion, citalopram, clomipramine, desipramine, doxepin, fluvoxamine, fluoxetine, imipramine, isocarboxazid, maprotiline, mirtazapine, nefazodone, nortriptyline, paroxetine, phenelzine, protriptyline, sertraline, tranlycypromine, trazodone, trimipramine, and venlafaxine), antianxiety agents (e.g., alprazolam, buspirone, chlordiazepoxide, clorazepate, diazepam, halazepam, lorazepam, oxazepam, and prazepam), and stimulants (e.g., d-amphetamine, methylphenidate, and pemoline).

In other embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with agents used to treat neurological disorders. Neurological agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, antiepileptic agents (e.g., carbamazepine, clonazepam, ethosuximide, phenobarbital, phenytoin, primidone, valproic acid, divalproex sodium, felbamate, gabapentin, lamotrigine, levetiracetam,

oxcarbazepine, tiagabine, topiramate, zonisamide, diazepam, lorazepam, and clonazepam), antiparkinsonian agents (e.g., levodopa/carbidopa, selegiline, amantidine, bromocriptine, pergolide, ropinirole, pramipexole, benzotropine; biperiden; ethopropazine; procyclidine; trihexyphenidyl, tolcapone), and ALS therapeutics (e.g. riluzole).

5 In another embodiment, albumin fusion proteins and/or polynucleotides of the invention are administered in combination with vasodilating agents and/or calcium channel blocking agents. Vasodilating agents that may be administered with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to, Angiotensin Converting Enzyme (ACE) inhibitors (e.g., papaverine, isoxsuprine, 10 benazepril, captopril, cilazapril, enalapril, enalaprilat, fosinopril, lisinopril, moexipril, perindopril, quinapril, ramipril, spirapril, trandolapril, and nylidrin), and nitrates (e.g., isosorbide dinitrate, isosorbide mononitrate, and nitroglycerin). Examples of calcium channel blocking agents that may be administered in combination with the albumin fusion proteins and/or polynucleotides of the invention include, but are not limited to amlodipine, 15 bepridil, diltiazem, felodipine, flunarizine, isradipine, nicardipine, nifedipine, nimodipine, and verapamil.

In certain embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with treatments for gastrointestinal disorders. Treatments for gastrointestinal disorders that may be administered with the albumin fusion 20 protein and/or polynucleotide of the invention include, but are not limited to, H<sub>2</sub> histamine receptor antagonists (e.g., TAGAMET<sup>TM</sup> (cimetidine), ZANTAC<sup>TM</sup> (ranitidine), PEPCID<sup>TM</sup> (famotidine), and AXID<sup>TM</sup> (nizatidine)); inhibitors of H<sup>+</sup>, K<sup>+</sup> ATPase (e.g., PREVACID<sup>TM</sup> (lansoprazole) and PRILOSEC<sup>TM</sup> (omeprazole)); Bismuth compounds (e.g., PEPTO-BISMOL<sup>TM</sup> (bismuth subsalicylate) and DE-NOL<sup>TM</sup> (bismuth subcitrate)); 25 various antacids; sucralfate; prostaglandin analogs (e.g. CYTOTEC<sup>TM</sup> (misoprostol)); muscarinic cholinergic antagonists; laxatives (e.g., surfactant laxatives, stimulant laxatives, saline and osmotic laxatives); antidiarrheal agents (e.g., LOMOTIL<sup>TM</sup> (diphenoxylate), MOTOFEN<sup>TM</sup> (diphenoxin), and IMODIUM<sup>TM</sup> (loperamide hydrochloride)), synthetic analogs of somatostatin such as SANDOSTATIN<sup>TM</sup> 30 (octreotide), antiemetic agents (e.g., ZOFRAN<sup>TM</sup> (ondansetron), KYTRIL<sup>TM</sup> (granisetron hydrochloride), tropisetron, dolasetron, metoclopramide, chlorpromazine, perphenazine, prochlorperazine, promethazine, thiethylperazine, triflupromazine, domperidone,



haloperidol, droperidol, trimethobenzamide, dexamethasone, methylprednisolone, dronabinol, and nabilone); D2 antagonists (e.g., metoclopramide, trimethobenzamide and chlorpromazine); bile salts; chenodeoxycholic acid; ursodeoxycholic acid; and pancreatic enzyme preparations such as pancreatin and pancrelipase.

5 In additional embodiments, the albumin fusion proteins and/or polynucleotides of the invention are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

The invention also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions comprising albumin fusion proteins of the invention. Optionally associated with such  
10 container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

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### **Gene Therapy**

Constructs encoding albumin fusion proteins of the invention can be used as a part of a gene therapy protocol to deliver therapeutically effective doses of the albumin fusion protein. A preferred approach for *in vivo* introduction of nucleic acid into a cell is by use  
20 of a viral vector containing nucleic acid, encoding an albumin fusion protein of the invention. Infection of cells with a viral vector has the advantage that a large proportion of the targeted cells can receive the nucleic acid. Additionally, molecules encoded within the viral vector, e.g., by a cDNA contained in the viral vector, are expressed efficiently in cells which have taken up viral vector nucleic acid.

25 Retrovirus vectors and adeno-associated virus vectors can be used as a recombinant gene delivery system for the transfer of exogenous nucleic acid molecules encoding albumin fusion proteins *in vivo*. These vectors provide efficient delivery of nucleic acids into cells, and the transferred nucleic acids are stably integrated into the chromosomal DNA of the host. The development of specialized cell lines (termed  
30 "packaging cells") which produce only replication-defective retroviruses has increased the utility of retroviruses for gene therapy, and defective retroviruses are characterized for use in gene transfer for gene therapy purposes (for a review see Miller, A.D. (1990) *Blood*

76:27 1). A replication defective retrovirus can be packaged into virions which can be used to infect a target cell through the use of a helper virus by standard techniques. Protocols for producing recombinant retroviruses and for infecting cells *in vitro* or *in vivo* with such viruses can be found in Current Protocols in Molecular Biology, Ausubel, F.M. 5 *et al.*, (eds.) Greene Publishing Associates, (1989), Sections 9.10-9.14 and other standard laboratory manuals.

Another viral gene delivery system useful in the present invention uses adenovirus-derived vectors. The genome of an adenovirus can be manipulated such that it encodes and expresses a gene product of interest but is inactivated in terms of its ability to 10 replicate in a normal lytic viral life cycle. See, for example, Berkner *et al.*, *BioTechniques* 6:616 (1988); Rosenfeld *et al.*, *Science* 252:431-434 (1991); and Rosenfeld *et al.*, *Cell* 68:143-155 (1992). Suitable adenoviral vectors derived from the adenovirus strain Ad type 5 d1324 or other strains of adenovirus (*e.g.*, Ad2, Ad3, Ad7 etc.) are known to those skilled in the art. Recombinant adenoviruses can be advantageous in certain 15 circumstances in that they are not capable of infecting nondividing cells and can be used to infect a wide variety of cell types, including epithelial cells (Rosenfeld *et al.*, (1992) cited supra). Furthermore, the virus particle is relatively stable and amenable to purification and concentration, and as above, can be modified so as to affect the spectrum of infectivity. Additionally, introduced adenoviral DNA (and foreign DNA contained therein) is not 20 integrated into the genome of a host cell but remains episomal, thereby avoiding potential problems that can occur as a result of insertional mutagenesis in situations where introduced DNA becomes integrated into the host genome (*e.g.*, retroviral DNA). Moreover, the carrying capacity of the adenoviral genome for foreign DNA is large (up to 8 kilobases) relative to other gene delivery vectors (Berkner *et al.*, cited supra; 25 Haj-Ahmand *et al.*, *J. Virol.* 57:267 (1986)).

In another embodiment, non-viral gene delivery systems of the present invention rely on endocytic pathways for the uptake of the subject nucleotide molecule by the targeted cell. Exemplary gene delivery systems of this type include liposomal derived systems, poly-lysine conjugates, and artificial viral envelopes. In a representative 30 embodiment, a nucleic acid molecule encoding an albumin fusion protein of the invention can be entrapped in liposomes bearing positive charges on their surface (*e.g.*, lipofectins) and (optionally) which are tagged with antibodies against cell surface antigens of the target

tissue (Mizuno *et al.* (1992) *No Shinkei Geka* 20:547-5 5 1; PCT publication W091/06309; Japanese patent application 1047381; and European patent publication EP-A-43075).

Gene delivery systems for a gene encoding an albumin fusion protein of the invention can be introduced into a patient by any of a number of methods. For instance, a pharmaceutical preparation of the gene delivery system can be introduced systemically, *e.g.* by intravenous injection, and specific transduction of the protein in the target cells occurs predominantly from specificity of transfection provided by the gene delivery vehicle, cell-type or tissue-type expression due to the transcriptional regulatory sequences controlling expression of the receptor gene, or a combination thereof. In other embodiments, initial delivery of the recombinant gene is more limited with introduction into the animal being quite localized. For example, the gene delivery vehicle can be introduced by catheter (see U.S. Patent 5,328,470) or by Stereotactic injection (*e.g.* Chen *et al.* (1994) *PNAS* 91: 3 054-3 05 7). The pharmaceutical preparation of the gene therapy construct can consist essentially of the gene delivery system in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Where the albumin fusion protein can be produced intact from recombinant cells, *e.g.* retroviral vectors, the pharmaceutical preparation can comprise one or more cells which produce the albumin fusion protein.

#### 20 *Additional Gene Therapy Methods*

Also encompassed by the invention are gene therapy methods for treating or preventing disorders, diseases and conditions. The gene therapy methods relate to the introduction of nucleic acid (DNA, RNA and antisense DNA or RNA) sequences into an animal to achieve expression of an albumin fusion protein of the invention. This method requires a polynucleotide which codes for an albumin fusion protein of the present invention operatively linked to a promoter and any other genetic elements necessary for the expression of the fusion protein by the target tissue. Such gene therapy and delivery techniques are known in the art, see, for example, WO90/11092, which is herein incorporated by reference.

Thus, for example, cells from a patient may be engineered with a polynucleotide (DNA or RNA) comprising a promoter operably linked to a polynucleotide encoding an albumin fusion protein of the present invention *ex vivo*, with the engineered cells then

being provided to a patient to be treated with the fusion protein of the present invention. Such methods are well-known in the art. For example, see Beldegrun, A., et al., J. Natl. Cancer Inst. 85: 207-216 (1993); Ferrantini, M. et al., Cancer Research 53: 1107-1112 (1993); Ferrantini, M. et al., J. Immunology 153: 4604-4615 (1994); Kaido, T., et al., Int. J. Cancer 60: 221-229 (1995); Ogura, H., et al., Cancer Research 50: 5102-5106 (1990); Santodonato, L., et al., Human Gene Therapy 7:1-10 (1996); Santodonato, L., et al., Gene Therapy 4:1246-1255 (1997); and Zhang, J.-F. et al., Cancer Gene Therapy 3: 31-38 (1996)), which are herein incorporated by reference. In one embodiment, the cells which are engineered are arterial cells. The arterial cells may be reintroduced into the patient through direct injection to the artery, the tissues surrounding the artery, or through catheter injection.

As discussed in more detail below, the polynucleotide constructs can be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of tissues (heart, muscle, skin, lung, liver, and the like). The polynucleotide constructs may be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

In one embodiment, polynucleotides encoding the albumin fusion proteins of the present invention is delivered as a naked polynucleotide. The term "naked" polynucleotide, DNA or RNA refers to sequences that are free from any delivery vehicle that acts to assist, promote or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating agents and the like. However, polynucleotides encoding the albumin fusion proteins of the present invention can also be delivered in liposome formulations and lipofectin formulations and the like can be prepared by methods well known to those skilled in the art. Such methods are described, for example, in U.S. Patent Nos. 5,593,972, 5,589,466, and 5,580,859, which are herein incorporated by reference.

The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Appropriate vectors include pWLNEO, pSV2CAT, pOG44, pXT1 and pSG available from Stratagene; pSVK3, pBPV, pMSG and pSVL available from Pharmacia; and pEF1/V5, pcDNA3.1, and pRc/CMV2 available from Invitrogen. Other suitable vectors will be readily apparent to the skilled artisan.

Any strong promoter known to those skilled in the art can be used for driving the expression of the polynucleotide sequence. Suitable promoters include adenoviral promoters, such as the adenoviral major late promoter; or heterologous promoters, such as the cytomegalovirus (CMV) promoter; the respiratory syncytial virus (RSV) promoter; 5 inducible promoters, such as the MMT promoter, the metallothionein promoter; heat shock promoters; the albumin promoter; the ApoAI promoter; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; the b-actin promoter; and human growth hormone promoters. The promoter also may be the native promoter for the gene corresponding to the Therapeutic 10 protein portion of the albumin fusion proteins of the invention.

Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to 15 six months.

The polynucleotide construct can be delivered to the interstitial space of tissues within the an animal, including of muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. 20 Interstitial space of the tissues comprises the intercellular, fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the 25 interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin 30 fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked nucleic acid sequence injection, an effective dosage amount of DNA

or RNA will be in the range of from about 0.05 mg/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection.

- 5 The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration.

The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as,  
10 inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked DNA constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The naked polynucleotides are delivered by any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous  
15 injection, topical administration, catheter infusion, and so-called "gene guns". These delivery methods are known in the art.

The constructs may also be delivered with delivery vehicles such as viral sequences, viral particles, liposome formulations, lipofectin, precipitating agents, etc. Such methods of delivery are known in the art.

20 In certain embodiments, the polynucleotide constructs are complexed in a liposome preparation. Liposomal preparations for use in the instant invention include cationic (positively charged), anionic (negatively charged) and neutral preparations. However, cationic liposomes are particularly preferred because a tight charge complex can be formed between the cationic liposome and the polyanionic nucleic acid. Cationic liposomes have  
25 been shown to mediate intracellular delivery of plasmid DNA (Felgner et al., Proc. Natl. Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference); mRNA (Malone et al., Proc. Natl. Acad. Sci. USA (1989) 86:6077-6081, which is herein incorporated by reference); and purified transcription factors (Debs et al., J. Biol. Chem. (1990) 265:10189-10192, which is herein incorporated by reference), in functional form.

30 Cationic liposomes are readily available. For example, N[1-2,3-dioleoyloxy)propyl]-N,N,N-triethylammonium (DOTMA) liposomes are particularly useful and are available under the trademark Lipofectin, from GIBCO BRL,

Grand Island, N.Y. (See, also, Felgner et al., Proc. Natl Acad. Sci. USA (1987) 84:7413-7416, which is herein incorporated by reference). Other commercially available liposomes include transfectace (DDAB/DOPE) and DOTAP/DOPE (Boehringer).

Other cationic liposomes can be prepared from readily available materials using techniques well known in the art. See, e.g. PCT Publication No. WO 90/11092 (which is herein incorporated by reference) for a description of the synthesis of DOTAP (1,2-bis(oleoyloxy)-3-(trimethylammonio)propane) liposomes. Preparation of DOTMA liposomes is explained in the literature, see, e.g., P. Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7417, which is herein incorporated by reference. Similar methods can be used to prepare liposomes from other cationic lipid materials.

Similarly, anionic and neutral liposomes are readily available, such as from Avanti Polar Lipids (Birmingham, Ala.), or can be easily prepared using readily available materials. Such materials include phosphatidyl, choline, cholesterol, phosphatidyl ethanolamine, dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), dioleoylphosphatidyl ethanolamine (DOPE), among others. These materials can also be mixed with the DOTMA and DOTAP starting materials in appropriate ratios. Methods for making liposomes using these materials are well known in the art.

For example, commercially dioleoylphosphatidyl choline (DOPC), dioleoylphosphatidyl glycerol (DOPG), and dioleoylphosphatidyl ethanolamine (DOPE) can be used in various combinations to make conventional liposomes, with or without the addition of cholesterol. Thus, for example, DOPG/DOPC vesicles can be prepared by drying 50 mg each of DOPG and DOPC under a stream of nitrogen gas into a sonication vial. The sample is placed under a vacuum pump overnight and is hydrated the following day with deionized water. The sample is then sonicated for 2 hours in a capped vial, using a Heat Systems model 350 sonicator equipped with an inverted cup (bath type) probe at the maximum setting while the bath is circulated at 15EC. Alternatively, negatively charged vesicles can be prepared without sonication to produce multilamellar vesicles or by extrusion through nucleopore membranes to produce unilamellar vesicles of discrete size. Other methods are known and available to those of skill in the art.

The liposomes can comprise multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), or large unilamellar vesicles (LUVs), with SUVs being preferred. The various liposome-nucleic acid complexes are prepared using methods well known in the

art. See, e.g., Straubinger et al., *Methods of Immunology* (1983), 101:512-527, which is herein incorporated by reference. For example, MLVs containing nucleic acid can be prepared by depositing a thin film of phospholipid on the walls of a glass tube and subsequently hydrating with a solution of the material to be encapsulated. SUVs are prepared by extended sonication of MLVs to produce a homogeneous population of unilamellar liposomes. The material to be entrapped is added to a suspension of preformed MLVs and then sonicated. When using liposomes containing cationic lipids, the dried lipid film is resuspended in an appropriate solution such as sterile water or an isotonic buffer solution such as 10 mM Tris/NaCl, sonicated, and then the preformed liposomes are mixed directly with the DNA. The liposome and DNA form a very stable complex due to binding of the positively charged liposomes to the cationic DNA. SUVs find use with small nucleic acid fragments. LUVs are prepared by a number of methods, well known in the art. Commonly used methods include  $\text{Ca}^{2+}$ -EDTA chelation (Papahadjopoulos et al., *Biochim. Biophys. Acta* (1975) 394:483; Wilson et al., *Cell* 17:77 (1979)); ether injection (Deamer, D. and Bangham, A., *Biochim. Biophys. Acta* 443:629 (1976); Ostro et al., *Biochem. Biophys. Res. Commun.* 76:836 (1977); Fraley et al., *Proc. Natl. Acad. Sci. USA* 76:3348 (1979)); detergent dialysis (Enoch, H. and Strittmatter, P., *Proc. Natl. Acad. Sci. USA* 76:145 (1979)); and reverse-phase evaporation (REV) (Fraley et al., *J. Biol. Chem.* 255:10431 (1980); Szoka, F. and Papahadjopoulos, D., *Proc. Natl. Acad. Sci. USA* 75:145 (1978); Schaefer-Ridder et al., *Science* 215:166 (1982)), which are herein incorporated by reference.

Generally, the ratio of DNA to liposomes will be from about 10:1 to about 1:10. Preferably, the ration will be from about 5:1 to about 1:5. More preferably, the ration will be about 3:1 to about 1:3. Still more preferably, the ratio will be about 1:1.

U.S. Patent No. 5,676,954 (which is herein incorporated by reference) reports on the injection of genetic material, complexed with cationic liposomes carriers, into mice. U.S. Patent Nos. 4,897,355, 4,946,787, 5,049,386, 5,459,127, 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 (which are herein incorporated by reference) provide cationic lipids for use in transfecting DNA into cells and mammals. U.S. Patent Nos. 5,589,466, 5,693,622, 5,580,859, 5,703,055, and international publication no. WO 94/9469 provide methods for delivering DNA-cationic lipid complexes to mammals.



In certain embodiments, cells are engineered, *ex vivo* or *in vivo*, using a retroviral particle containing RNA which comprises a sequence encoding an albumin fusion protein of the present invention. Retroviruses from which the retroviral plasmid vectors may be derived include, but are not limited to, Moloney Murine Leukemia Virus, spleen necrosis virus, Rous sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, gibbon ape leukemia virus, human immunodeficiency virus, Myeloproliferative Sarcoma Virus, and mammary tumor virus.

The retroviral plasmid vector is employed to transduce packaging cell lines to form producer cell lines. Examples of packaging cells which may be transfected include, but are not limited to, the PE501, PA317, R-2, R-AM, PA12, T19-14X, VT-19-17-H2, RCRE, RCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy 1:5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO<sub>4</sub> precipitation. In one alternative, the retroviral plasmid vector may be encapsulated into a liposome, or coupled to a lipid, and then administered to a host.

The producer cell line generates infectious retroviral vector particles which include polynucleotide encoding an albumin fusion protein of the present invention. Such retroviral vector particles then may be employed, to transduce eukaryotic cells, either *in vitro* or *in vivo*. The transduced eukaryotic cells will express a fusion protein of the present invention.

In certain other embodiments, cells are engineered, *ex vivo* or *in vivo*, with polynucleotide contained in an adenovirus vector. Adenovirus can be manipulated such that it encodes and expresses fusion protein of the present invention, and at the same time is inactivated in terms of its ability to replicate in a normal lytic viral life cycle. Adenovirus expression is achieved without integration of the viral DNA into the host cell chromosome, thereby alleviating concerns about insertional mutagenesis. Furthermore, adenoviruses have been used as live enteric vaccines for many years with an excellent safety profile (Schwartz et al. Am. Rev. Respir. Dis.109:233-238 (1974)). Finally, adenovirus mediated gene transfer has been demonstrated in a number of instances including transfer of alpha-1-antitrypsin and CFTR to the lungs of cotton rats (Rosenfeld, M. A. et al. (1991) Science 252:431-434; Rosenfeld et al., (1992) Cell 68:143-155).

Furthermore, extensive studies to attempt to establish adenovirus as a causative agent in human cancer were uniformly negative (Green, M. et al. (1979) Proc. Natl. Acad. Sci. USA 76:6606).

Suitable adenoviral vectors useful in the present invention are described, for example, in Kozarsky and Wilson, Curr. Opin. Genet. Devel. 3:499-503 (1993); Rosenfeld et al., Cell 68:143-155 (1992); Engelhardt et al., Human Genet. Ther. 4:759-769 (1993); Yang et al., Nature Genet. 7:362-369 (1994); Wilson et al., Nature 365:691-692 (1993); and U.S. Patent No. 5,652,224, which are herein incorporated by reference. For example, the adenovirus vector Ad2 is useful and can be grown in human 293 cells. These cells contain the E1 region of adenovirus and constitutively express Ela and Elb, which complement the defective adenoviruses by providing the products of the genes deleted from the vector. In addition to Ad2, other varieties of adenovirus (e.g., Ad3, Ad5, and Ad7) are also useful in the present invention.

Preferably, the adenoviruses used in the present invention are replication deficient. Replication deficient adenoviruses require the aid of a helper virus and/or packaging cell line to form infectious particles. The resulting virus is capable of infecting cells and can express a polynucleotide of interest which is operably linked to a promoter, but cannot replicate in most cells. Replication deficient adenoviruses may be deleted in one or more of all or a portion of the following genes: E1a, E1b, E3, E4, E2a, or L1 through L5.

In certain other embodiments, the cells are engineered, ex vivo or *in vivo*, using an adeno-associated virus (AAV). AAVs are naturally occurring defective viruses that require helper viruses to produce infectious particles (Muzyczka, N., Curr. Topics in Microbiol. Immunol. 158:97 (1992)). It is also one of the few viruses that may integrate its DNA into non-dividing cells. Vectors containing as little as 300 base pairs of AAV can be packaged and can integrate, but space for exogenous DNA is limited to about 4.5 kb. Methods for producing and using such AAVs are known in the art. See, for example, U.S. Patent Nos. 5,139,941, 5,173,414, 5,354,678, 5,436,146, 5,474,935, 5,478,745, and 5,589,377.

For example, an appropriate AAV vector for use in the present invention will include all the sequences necessary for DNA replication, encapsidation, and host-cell integration. The polynucleotide construct is inserted into the AAV vector using standard cloning methods, such as those found in Sambrook et al., Molecular Cloning: A

Laboratory Manual, Cold Spring Harbor Press (1989). The recombinant AAV vector is then transfected into packaging cells which are infected with a helper virus, using any standard technique, including lipofection, electroporation, calcium phosphate precipitation, etc. Appropriate helper viruses include adenoviruses, cytomegaloviruses, vaccinia viruses, or herpes viruses. Once the packaging cells are transfected and infected, they will produce infectious AAV viral particles which contain the polynucleotide construct. These viral particles are then used to transduce eukaryotic cells, either *ex vivo* or *in vivo*. The transduced cells will contain the polynucleotide construct integrated into its genome, and will express a fusion protein of the invention.

Another method of gene therapy involves operably associating heterologous control regions and endogenous polynucleotide sequences (e.g. encoding a polypeptide of the present invention) via homologous recombination (see, e.g., U.S. Patent No. 5,641,670, issued June 24, 1997; International Publication No. WO 96/29411, published September 26, 1996; International Publication No. WO 94/12650, published August 4, 1994; Koller et al., Proc. Natl. Acad. Sci. USA 86:8932-8935 (1989); and Zijlstra et al., Nature 342:435-438 (1989), which are herein incorporated by reference. This method involves the activation of a gene which is present in the target cells, but which is not normally expressed in the cells, or is expressed at a lower level than desired.

Polynucleotide constructs are made, using standard techniques known in the art, which contain the promoter with targeting sequences flanking the promoter. Suitable promoters are described herein. The targeting sequence is sufficiently complementary to an endogenous sequence to permit homologous recombination of the promoter-targeting sequence with the endogenous sequence. The targeting sequence will be sufficiently near the 5' end of the desired endogenous polynucleotide sequence so the promoter will be operably linked to the endogenous sequence upon homologous recombination.

The promoter and the targeting sequences can be amplified using PCR. Preferably, the amplified promoter contains distinct restriction enzyme sites on the 5' and 3' ends. Preferably, the 3' end of the first targeting sequence contains the same restriction enzyme site as the 5' end of the amplified promoter and the 5' end of the second targeting sequence contains the same restriction site as the 3' end of the amplified promoter. The amplified promoter and targeting sequences are digested and ligated together.

The promoter-targeting sequence construct is delivered to the cells, either as naked

polynucleotide, or in conjunction with transfection-facilitating agents, such as liposomes, viral sequences, viral particles, whole viruses, lipofection, precipitating agents, etc., described in more detail above. The P promoter-targeting sequence can be delivered by any method, included direct needle injection, intravenous injection, topical administration, catheter infusion, particle accelerators, etc. The methods are described in more detail below.

The promoter-targeting sequence construct is taken up by cells. Homologous recombination between the construct and the endogenous sequence takes place, such that an endogenous sequence is placed under the control of the promoter. The promoter then drives the expression of the endogenous sequence.

The polynucleotide encoding an albumin fusion protein of the present invention may contain a secretory signal sequence that facilitates secretion of the protein. Typically, the signal sequence is positioned in the coding region of the polynucleotide to be expressed towards or at the 5' end of the coding region. The signal sequence may be homologous or heterologous to the polynucleotide of interest and may be homologous or heterologous to the cells to be transfected. Additionally, the signal sequence may be chemically synthesized using methods known in the art.

Any mode of administration of any of the above-described polynucleotides constructs can be used so long as the mode results in the expression of one or more molecules in an amount sufficient to provide a therapeutic effect. This includes direct needle injection, systemic injection, catheter infusion, biolistic injectors, particle accelerators (i.e., "gene guns"), gelfoam sponge depots, other commercially available depot materials, osmotic pumps (e.g., Alza minipumps), oral or suppositorial solid (tablet or pill) pharmaceutical formulations, and decanting or topical applications during surgery.

For example, direct injection of naked calcium phosphate-precipitated plasmid into rat liver and rat spleen or a protein-coated plasmid into the portal vein has resulted in gene expression of the foreign gene in the rat livers (Kaneda et al., Science 243:375 (1989)).

A preferred method of local administration is by direct injection. Preferably, an albumin fusion protein of the present invention complexed with a delivery vehicle is administered by direct injection into or locally within the area of arteries. Administration of a composition locally within the area of arteries refers to injecting the composition centimeters and preferably, millimeters within arteries.

Another method of local administration is to contact a polynucleotide construct of the present invention in or around a surgical wound. For example, a patient can undergo surgery and the polynucleotide construct can be coated on the surface of tissue inside the wound or the construct can be injected into areas of tissue inside the wound.

5       Therapeutic compositions useful in systemic administration, include fusion proteins of the present invention complexed to a targeted delivery vehicle of the present invention. Suitable delivery vehicles for use with systemic administration comprise liposomes comprising ligands for targeting the vehicle to a particular site. In specific embodiments, suitable delivery vehicles for use with systemic administration comprise  
10       liposomes comprising albumin fusion proteins of the invention for targeting the vehicle to a particular site.

Preferred methods of systemic administration, include intravenous injection, aerosol, oral and percutaneous (topical) delivery. Intravenous injections can be performed using methods standard in the art. Aerosol delivery can also be performed using methods  
15       standard in the art (see, for example, Stribling et al., Proc. Natl. Acad. Sci. USA 189:11277-11281, 1992, which is incorporated herein by reference). Oral delivery can be performed by complexing a polynucleotide construct of the present invention to a carrier capable of withstanding degradation by digestive enzymes in the gut of an animal. Examples of such carriers, include plastic capsules or tablets, such as those known in the  
20       art. Topical delivery can be performed by mixing a polynucleotide construct of the present invention with a lipophilic reagent (e.g., DMSO) that is capable of passing into the skin.

Determining an effective amount of substance to be delivered can depend upon a number of factors including, for example, the chemical structure and biological activity of the substance, the age and weight of the animal, the precise condition requiring treatment  
25       and its severity, and the route of administration. The frequency of treatments depends upon a number of factors, such as the amount of polynucleotide constructs administered per dose, as well as the health and history of the subject. The precise amount, number of doses, and timing of doses will be determined by the attending physician or veterinarian.

Albumin fusion proteins of the present invention can be administered to any  
30       animal, preferably to mammals and birds. Preferred mammals include humans, dogs, cats, mice, rats, rabbits sheep, cattle, horses and pigs, with humans being particularly preferred.

### **Biological Activities**

Albumin fusion proteins and/or polynucleotides encoding albumin fusion proteins of the present invention, can be used in assays to test for one or more biological activities.

- 5 If an albumin fusion protein and/or polynucleotide exhibits an activity in a particular assay, it is likely that the Therapeutic protein corresponding to the fusion protein may be involved in the diseases associated with the biological activity. Thus, the fusion protein could be used to treat the associated disease.

- 10 Members of the secreted family of proteins are believed to be involved in biological activities associated with, for example, cellular signaling. Accordingly, albumin fusion proteins of the invention and polynucleotides encoding these proteins, may be used in diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders associated with aberrant activity of secreted polypeptides.

- 15 In preferred embodiments, fusion proteins of the present invention may be used in the diagnosis, prognosis, prevention and/or treatment of diseases and/or disorders relating to diseases and disorders of the endocrine system, the nervous system (See, for example, "Neurological Disorders" section below), and the immune system (See, for example, "Immune Activity" section below), respiratory system (See, for example, "Respiratory Disorders" section below), cardiovascular system (See, for example, "Cardiovascular Disorders" section below), reproductive system (See, for example, "Reproductive System Disorders" section below) digestive system (See, for example, "Gastrointestinal Disorders" section below), diseases and/or disorders relating to cell proliferation (See, for example, "Hyperproliferative Disorders" section below), and/or diseases or disorders relating to the blood ((See, for example, "Blood-Related Disorders" section below).

- 25 In preferred embodiments, the present invention encompasses a method of treating a disease or disorder listed in the "Preferred Indication Y" column of Table 1 comprising administering to a patient in which such treatment, prevention or amelioration is desired an albumin fusion protein of the invention that comprises a Therapeutic protein portion corresponding to a Therapeutic protein disclosed in the "Therapeutic Protein X" column of  
30 Table 1 (in the same row as the disease or disorder to be treated is listed in the "Preferred Indication Y" column of Table 1) in an amount effective to treat, prevent or ameliorate the disease or disorder.

In certain embodiments, a Therapeutic protein having a "Cancer" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to a Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., leukemia, cancer, and/or as described below under "Hyperproliferative Disorders").

In additional embodiments, a Therapeutic protein having a "Cancer" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a neoplasm located in a tissue selected from the group consisting of: colon, abdomen, bone, breast, digestive system, liver, pancreas, prostate, peritoneum, lung, blood (e.g., leukemia), endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), uterus, eye, head and neck, nervous (central and peripheral), lymphatic system, pelvic, skin, soft tissue, spleen, thoracic, and urogenital.

In other embodiments, a Therapeutic protein having a "Cancer" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a pre-neoplastic condition, selected from the group consisting of: hyperplasia (e.g., endometrial hyperplasia and/or as described in the section entitled "Hyperproliferative Disorders"), metaplasia (e.g., connective tissue metaplasia, atypical metaplasia, and/or as described in the section entitled "Hyperproliferative Disorders"), and dysplasia (e.g., cervical dysplasia, and bronchopulmonary dysplasia).

In additional embodiments, a Therapeutic protein having a "Cancer" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a benign dysproliferative disorder selected from the group consisting of: benign tumors, fibrocystic conditions, tissue hypertrophy, and/or as described in the section entitled "Hyperproliferative Disorders".

In certain embodiments, a Therapeutic protein having a "Immune/Hematopoietic" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and

fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders"), a blood disorder (e.g., as described below under "Immune Activity", "Cardiovascular Disorders" and/or "Blood-Related Disorders"), and/or an infection (e.g., as described below under "Infectious Disease").

In additional embodiments, a Therapeutic protein having a "Immune/Hematopoietic" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: anemia, pancytopenia, leukopenia, thrombocytopenia, leukemias, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL), plasmacytomas, multiple myeloma, Burkitt's lymphoma, arthritis, asthma, AIDS, autoimmune disease, rheumatoid arthritis, granulomatous disease, immune deficiency, inflammatory bowel disease, sepsis, neutropenia, neutrophilia, psoriasis, an immune reaction to a transplanted organ and/or tissue, systemic lupus erythematosus, hemophilia, hypercoagulation, diabetes mellitus, endocarditis, meningitis, Lyme Disease, and allergies.

In other embodiments, a Therapeutic protein having a "Reproductive" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders"), and/or a disorder of the reproductive system (e.g., as described below under "Reproductive System Disorders").

In additional embodiments, a Therapeutic protein having a "Reproductive" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: cryptorchism, prostatitis, inguinal hernia, varicocele, a leydig cell tumor, verrucous carcinoma, prostatitis, malacoplakia, Peyronie's disease, penile carcinoma, squamous cell hyperplasia, dysmenorrhea, ovarian adenocarcinoma, Turner's syndrome, mucopurulent cervicitis, a Sertoli-leydig tumor, ovarian cancer, uterine cancer,



pelvic inflammatory disease, testicular cancer, prostate cancer, Klinefelter's syndrome, Young's syndrome, premature ejaculation, diabetes mellitus, cystic fibrosis, Kartagener's syndrome, testicular atrophy, testicular feminization, anorchia, ectopic testis, epididymitis, orchitis, gonorrhea, syphilis, testicular torsion, vasitis nodosa, a germ cell tumor, a stromal tumor, dysmenorrhea, retroverted uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhea, Cushing's syndrome, a hydatidiform mole, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding, cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, a cervical neoplasm, pseudohermaphroditism, and premenstrual syndrome.

In other embodiments, a Therapeutic protein having a "Musculoskeletal" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders"), and/or a disorder of the immune system (e.g., as described below under "Immune Activity").

In further embodiments, a Therapeutic protein having a "Musculoskeletal" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: bone cancer (e.g., osteochondroma, benign chondroma, chondroblastoma, chondromyxoid fibroma, osteoid osteoma, giant cell tumor, multiple myeloma, and osteosarcoma), Paget's Disease, rheumatoid arthritis, systemic lupus erythematosus, osteomyelitis, Lyme Disease, gout, bursitis, tendonitis, osteoporosis, osteoarthritis, muscular dystrophy, mitochondrial myopathy, cachexia, and multiple sclerosis.

In other embodiments, a Therapeutic protein having a "Cardiovascular" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders"), and/or a disorder of the cardiovascular system (e.g., as described below under "Cardiovascular Disorders").

In additional embodiments, a Therapeutic protein having a "Cardiovascular" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: myxoma, fibroma, rhabdomyoma, cardiovascular abnormality (e.g., a congenital heart defect, cerebral arteriovenous malformation, septal defect), heart disease (e.g., heart failure, congestive heart disease, arrhythmia, tachycardia, fibrillation, pericardial Disease, endocarditis), cardiac arrest, heart valve disease (e.g., stenosis, regurgitation, prolapse), vascular disease (e.g., hypertension, coronary artery disease, angina, aneurysm, arteriosclerosis, peripheral vascular disease), hyponatremia, hypernatremia, hypokalemia, and hyperkalemia.

In other embodiments, a Therapeutic protein having a "Mixed Fetal" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders").

In further embodiments, a Therapeutic protein having a "Mixed Fetal" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: spina bifida, hydranencephaly, neurofibromatosis, fetal alcohol syndrome, diabetes mellitus, PKU, Down's syndrome, Patau syndrome, Edwards syndrome, Turner syndrome, Apert syndrome, Carpenter syndrome, Conradi syndrome, Crouzon syndrome, cutis laxa, Cornelia de Lange syndrome, Ellis-van Creveld syndrome, Holt-Oram syndrome, Kartagener syndrome, Meckel-Gruber syndrome, Noonan syndrome, Pallister-Hall syndrome, Rubinstein-Taybi syndrome, Scimitar syndrome, Smith-Lemli-Opitz syndrome, thrombocytopenia-absent radius (TAR) syndrome, Treacher Collins syndrome, Williams syndrome, Hirschsprung's disease, Meckel's diverticulum, polycystic kidney disease, Turner's syndrome, and gonadal dysgenesis, Klippel-Feil syndrome, Osteogenesis imperfecta, muscular dystrophy, Tay-Sachs disease, Wilm's tumor, neuroblastoma, and retinoblastoma.

In further embodiments, a Therapeutic protein having a "Excretory" recitation in

the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders") and/or a renal disorder (e.g., as described below under "Renal Disorders").

In other embodiments, a Therapeutic protein having a "Excretory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: bladder cancer, prostate cancer, benign prostatic hyperplasia, bladder disorders (e.g., urinary incontinence, urinary retention, urinary obstruction, urinary tract infections, interstitial cystitis, prostatitis, neurogenic bladder, hematuria), a renal disorder (e.g., hydronephrosis, proteinuria, renal failure, pyelonephritis, urolithiasis, reflux nephropathy, and unilateral obstructive uropathy).

In further embodiments, a Therapeutic protein having a "Neural/Sensory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders") and/or a disease or disorder of the nervous system (e.g., as described below under "Neural Activity and Neurological Diseases").

In other embodiments, a Therapeutic protein having a "Neural/Sensory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: brain cancer (e.g., brain stem glioma, brain tumor, central nervous system (Primary) lymphoma, central nervous system lymphoma, cerebellar astrocytoma, and cerebral astrocytoma, a neurodegenerative disorder (e.g., Alzheimer's Disease, Creutzfeldt-Jakob Disease, Parkinson's Disease, and Idiopathic Presenile Dementia), encephalomyelitis, cerebral malaria, meningitis, a metabolic brain disease (e.g., phenylketonuria and pyruvate carboxylase deficiency), cerebellar ataxia, ataxia telangiectasia, and AIDS Dementia Complex, schizophrenia, attention deficit disorder,

hyperactive attention deficit disorder, autism, and an obsessive compulsive disorder.

In other embodiments, a Therapeutic protein having a "Respiratory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders") and/or a disease or disorder of the respiratory system (e.g., as described below under "Respiratory Disorders").

In other embodiments, a Therapeutic protein having a "Respiratory" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: a cancer of the respiratory system (such as larynx cancer, pharynx cancer, trachea cancer, epiglottis cancer, lung cancer, squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma), an allergic reaction, cystic fibrosis, sarcoidosis, histiocytosis X, an infiltrative lung disease (e.g., pulmonary fibrosis and lymphoid interstitial pneumonia), an obstructive airway disease (e.g., asthma, emphysema, chronic or acute bronchitis), an occupational lung disease (e.g., silicosis and asbestosis), pneumonia, and pleurisy.

In other embodiments, a Therapeutic protein having an "Endocrine" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders"), a disease or disorder of the respiratory system (e.g., as described below under "Respiratory Disorders"), a renal disorder (e.g., as described below under "Renal Disorders"), and/or a disorder of the endocrine system (e.g., as described below under "Endocrine Disorders").

In other embodiments, a Therapeutic protein having a "Endocrine" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: a cancer of endocrine tissues and/or organs (e.g., cancer of the

- hypothalamus, pituitary gland, thyroid gland, parathyroid glands, pancreas, adrenal glands, ovaries, and testes), diabetes (e.g., diabetes insipidus, type I and type II diabetes mellitus), obesity, a disorder related to pituitary glands (e.g., hyperpituitarism, hypopituitarism, and pituitary dwarfism), hypothyroidism, hyperthyroidism, goiter, reproductive disorders (e.g. male and female infertility), a disorder related to adrenal glands (e.g., Addison's Disease, corticosteroid deficiency, and Cushing's Syndrome), kidney cancer (e.g., hypernephroma, transitional cell cancer, and Wilm's tumor), diabetic nephropathy, interstitial nephritis, polycystic kidney disease, glomerulonephritis (e.g., IgM mesangial proliferative glomerulonephritis and glomerulonephritis caused by an autoimmune disorder; such as Goodpasture's syndrome), and nephrocalcinosis.

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In additional embodiments, a Therapeutic protein having a "Digestive" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to, for example, a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders") and/or a disease or disorder of the gastrointestinal system (e.g., as described below under "Gastrointestinal Disorders").

In other embodiments, a Therapeutic protein having a "Digestive" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: ulcerative colitis, appendicitis, Crohn's disease, hepatitis, hepatic encephalopathy, portal hypertension, cholelithiasis, cancer of the digestive system (e.g., biliary tract cancer, stomach cancer, colon cancer, gastric cancer, pancreatic cancer, cancer of the bile duct, a tumor of the colon (e.g., polyps or cancers), and cirrhosis), pancreatitis, ulcerative disease, pyloric stenosis, gastroenteritis, gastritis, gastric atrophy, a benign tumor of the duodenum, distension, irritable bowel syndrome, malabsorption, a congenital disorder of the small intestine, bacterial and parasitic infection, megacolon, Hirschsprung's disease, aganglionic megacolon, acquired megacolon, colitis, a anorectal disorder (e.g., anal fistulas, hemorrhoids), a congenital disorder of the liver (e.g., Wilson's disease, hemochromatosis, cystic fibrosis, biliary atresia, and alpha-1-antitrypsin deficiency), portal hypertension, cholelithiasis, and jaundice.

In further embodiments, a Therapeutic protein having a "Connective/Epithelial" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder relating to a neoplastic disease (e.g., as described below under "Hyperproliferative Disorders"), a cellular and/or genetic abnormality (e.g., as described below under "Diseases at the Cellular Level"), angiogenesis (e.g., as described below under "Anti-Angiogenesis Activity"), and/or to promote or inhibit regeneration (e.g., as described below under "Regeneration"), and/or wound healing (e.g., as described below under "Wound Healing and Epithelial Cell Proliferation").

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In certain embodiments, a Therapeutic protein having a "Connective/Epithelial" recitation in the "Preferred Indication" column of Table 1, an albumin fusion protein that comprises a Therapeutic protein portion corresponding to this Therapeutic protein, and fragments and variants thereof, may be used to treat a disease and/or disorder selected from the group consisting of: connective tissue metaplasia, mixed connective tissue disease, focal epithelial hyperplasia, epithelial metaplasia, mucoepithelial dysplasia, graft v. host disease, polymyositis, cystic hyperplasia, cerebral dysplasia, tissue hypertrophy, Alzheimer's disease, lymphoproliferative disorder, Waldenström's macroglobulinemia, Crohn's disease, pernicious anemia, idiopathic Addison's disease, glomerulonephritis, bullous pemphigoid, Sjögren's syndrome, diabetes mellitus, cystic fibrosis, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, osteoporosis, osteoarthritis, periodontal disease, wound healing, relapsing polychondritis, vasculitis, polyarteritis nodosa, Wegener's granulomatosis, cellulitis, rheumatoid arthritis, psoriatic arthritis, discoid lupus erythematosus, systemic lupus erythematosus, scleroderma, CREST syndrome, Sjögren's syndrome, polymyositis, dermatomyositis, mixed connective tissue disease, relapsing polychondritis, vasculitis, Henoch-Schönlein syndrome, erythema nodosum, polyarteritis nodosa, temporal (giant cell) arteritis, Takayasu's arteritis, Wegener's granulomatosis, Reiter's syndrome, Behçet's syndrome, ankylosing spondylitis, cellulitis, keloids, Ehler Danlos syndrome, Marfan syndrome, pseudoxanthoma elasticum, osteogenesis imperfecta, chondrodysplasias, epidermolysis bullosa, Alport syndrome, and cutis laxa.

In certain embodiments, an albumin fusion protein of the present invention may be used to diagnose and/or prognose diseases and/or disorders associated with the tissue(s) in which the gene corresponding to the Therapeutic protein portion of the fusion portion of the invention is expressed.

Thus, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention are useful in the diagnosis, detection and/or treatment of diseases and/or disorders associated with activities that include, but are not limited to, prohormone activation, neurotransmitter activity, cellular signaling, cellular proliferation, cellular differentiation, and cell migration.

More generally, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may be useful for the diagnosis, prognosis,

prevention and/or treatment of diseases and/or disorders associated with the following systems.

### **Immune Activity**

5 Albumin fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, diagnosing and/or prognosing diseases, disorders, and/or conditions of the immune system, by, for example, activating or inhibiting the proliferation, differentiation, or mobilization (chemotaxis) of immune cells. Immune cells develop through a process called hematopoiesis, producing  
10 myeloid (platelets, red blood cells, neutrophils, and macrophages) and lymphoid (B and T lymphocytes) cells from pluripotent stem cells. The etiology of these immune diseases, disorders, and/or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy or toxins), or infectious. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin  
15 fusion proteins of the invention can be used as a marker or detector of a particular immune system disease or disorder.

In another embodiment, a fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention, may be used to treat diseases and disorders of the immune system and/or to inhibit or enhance an immune response  
20 generated by cells associated with the tissue(s) in which the polypeptide of the invention is expressed.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, diagnosing, and/or prognosing immunodeficiencies, including both congenital and acquired  
25 immunodeficiencies. Examples of B cell immunodeficiencies in which immunoglobulin levels B cell function and/or B cell numbers are decreased include: X-linked agammaglobulinemia (Bruton's disease), X-linked infantile agammaglobulinemia, X-linked immunodeficiency with hyper IgM, non X-linked immunodeficiency with hyper IgM, X-linked lymphoproliferative syndrome (XLP), agammaglobulinemia including  
30 congenital and acquired agammaglobulinemia, adult onset agammaglobulinemia, late-onset agammaglobulinemia, dysgammaglobulinemia, hypogammaglobulinemia, unspecified hypogammaglobulinemia, recessive agammaglobulinemia (Swiss type),



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Selective IgM deficiency, selective IgA deficiency, selective IgG subclass deficiencies, IgG subclass deficiency (with or without IgA deficiency), Ig deficiency with increased IgM, IgG and IgA deficiency with increased IgM, antibody deficiency with normal or elevated Igs, Ig heavy chain deletions, kappa chain deficiency, B cell lymphoproliferative disorder (BLPD), common variable immunodeficiency (CVID), common variable immunodeficiency (CVI) (acquired), and transient hypogammaglobulinemia of infancy.

In specific embodiments, ataxia-telangiectasia or conditions associated with ataxia-telangiectasia are treated, prevented, diagnosed, and/or prognosing using the, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

Examples of congenital immunodeficiencies in which T cell and/or B cell function and/or number is decreased include, but are not limited to: DiGeorge anomaly, severe combined immunodeficiencies (SCID) (including, but not limited to, X-linked SCID, autosomal recessive SCID, adenosine deaminase deficiency, purine nucleoside phosphorylase (PNP) deficiency, Class II MHC deficiency (Bare lymphocyte syndrome), Wiskott-Aldrich syndrome, and ataxia telangiectasia), thymic hypoplasia, third and fourth pharyngeal pouch syndrome, 22q11.2 deletion, chronic mucocutaneous candidiasis, natural killer cell deficiency (NK), idiopathic CD4+ T-lymphocytopenia, immunodeficiency with predominant T cell defect (unspecified), and unspecified immunodeficiency of cell mediated immunity.

In specific embodiments, DiGeorge anomaly or conditions associated with DiGeorge anomaly are treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

Other immunodeficiencies that may be treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, chronic granulomatous disease, Chédiak-Higashi syndrome, myeloperoxidase deficiency, leukocyte glucose-6-phosphate dehydrogenase deficiency, X-linked lymphoproliferative syndrome (XLP), leukocyte adhesion deficiency, complement component deficiencies (including C1, C2, C3, C4, C5, C6, C7, C8 and/or C9 deficiencies), reticular dysgenesis, thymic aplasia, immunodeficiency with thymoma, severe congenital leukopenia,

dysplasia with immunodeficiency, neonatal neutropenia, short limbed dwarfism, and Nezelof syndrome-combined immunodeficiency with Igs.

In a preferred embodiment, the immunodeficiencies and/or conditions associated with the immunodeficiencies recited above are treated, prevented, diagnosed and/or  
5 prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

In a preferred embodiment fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used as an agent to boost immunoresponsiveness among immunodeficient individuals. In specific embodiments,  
10 fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used as an agent to boost immunoresponsiveness among B cell and/or T cell immunodeficient individuals.

The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, diagnosing  
15 and/or prognosing autoimmune disorders. Many autoimmune disorders result from inappropriate recognition of self as foreign material by immune cells. This inappropriate recognition results in an immune response leading to the destruction of the host tissue. Therefore, the administration of fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention that can inhibit an immune response,  
20 particularly the proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing autoimmune disorders.

Autoimmune diseases or disorders that may be treated, prevented, diagnosed and/or prognosed by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, one or more of the  
25 following: systemic lupus erythematosus, rheumatoid arthritis, ankylosing spondylitis, multiple sclerosis, autoimmune thyroiditis, Hashimoto's thyroiditis, autoimmune hemolytic anemia, hemolytic anemia, thrombocytopenia, autoimmune thrombocytopenia purpura, autoimmune neonatal thrombocytopenia, idiopathic thrombocytopenia purpura, purpura (e.g., Henloch-Scoenlein purpura), autoimmunocytopenia, Goodpasture's  
30 syndrome, Pemphigus vulgaris, myasthenia gravis, Grave's disease (hyperthyroidism), and insulin-resistant diabetes mellitus.

Additional disorders that are likely to have an autoimmune component that may be

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treated, prevented, and/or diagnosed with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, type II collagen-induced arthritis, antiphospholipid syndrome, dermatitis, allergic encephalomyelitis, myocarditis, relapsing polychondritis, rheumatic heart disease, neuritis, uveitis ophthalmia, polyendocrinopathies, Reiter's Disease, Stiff-Man Syndrome, autoimmune pulmonary inflammation, autism, Guillain-Barre Syndrome, insulin dependent diabetes mellitus, and autoimmune inflammatory eye disorders.

Additional disorders that are likely to have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, scleroderma with anti-collagen antibodies (often characterized, e.g., by nucleolar and other nuclear antibodies), mixed connective tissue disease (often characterized, e.g., by antibodies to extractable nuclear antigens (e.g., ribonucleoprotein)), polymyositis (often characterized, e.g., by nonhistone ANA), pernicious anemia (often characterized, e.g., by antiparietal cell, microsomes, and intrinsic factor antibodies), idiopathic Addison's disease (often characterized, e.g., by humoral and cell-mediated adrenal cytotoxicity, infertility (often characterized, e.g., by antispermatozoal antibodies), glomerulonephritis (often characterized, e.g., by glomerular basement membrane antibodies or immune complexes), bullous pemphigoid (often characterized, e.g., by IgG and complement in basement membrane), Sjogren's syndrome (often characterized, e.g., by multiple tissue antibodies, and/or a specific nonhistone ANA (SS-B)), diabetes mellitus (often characterized, e.g., by cell-mediated and humoral islet cell antibodies), and adrenergic drug resistance (including adrenergic drug resistance with asthma or cystic fibrosis) (often characterized, e.g., by beta-adrenergic receptor antibodies).

Additional disorders that may have an autoimmune component that may be treated, prevented, diagnosed and/or prognosed with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, chronic active hepatitis (often characterized, e.g., by smooth muscle antibodies), primary biliary cirrhosis (often characterized, e.g., by mitochondria antibodies), other endocrine gland failure (often characterized, e.g., by specific tissue antibodies in some cases), vitiligo (often characterized, e.g., by melanocyte antibodies),

vasculitis (often characterized, e.g., by Ig and complement in vessel walls and/or low serum complement), post-MI (often characterized, e.g., by myocardial antibodies), cardiomy syndrome (often characterized, e.g., by myocardial antibodies), urticaria (often characterized, e.g., by IgG and IgM antibodies to IgE), atopic dermatitis (often characterized, e.g., by IgG and IgM antibodies to IgE), asthma (often characterized, e.g., by IgG and IgM antibodies to IgE), and many other inflammatory, granulomatous, degenerative, and atrophic disorders.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using for example, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. In a specific preferred embodiment, rheumatoid arthritis is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

In another specific preferred embodiment, systemic lupus erythematosus is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. In another specific preferred embodiment, idiopathic thrombocytopenia purpura is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

In another specific preferred embodiment IgA nephropathy is treated, prevented, and/or diagnosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

In a preferred embodiment, the autoimmune diseases and disorders and/or conditions associated with the diseases and disorders recited above are treated, prevented, diagnosed and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention.

In preferred embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a immunosuppressive agent(s).

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, prognosing, and/or

diagnosing diseases, disorders, and/or conditions of hematopoietic cells. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with a decrease in certain (or many) types of hematopoietic cells, including but not limited to, leukopenia, neutropenia, anemia, and thrombocytopenia. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used to increase differentiation and proliferation of hematopoietic cells, including the pluripotent stem cells, in an effort to treat or prevent those diseases, disorders, and/or conditions associated with an increase in certain (or many) types of hematopoietic cells, including but not limited to, histiocytosis.

Allergic reactions and conditions, such as asthma (particularly allergic asthma) or other respiratory problems, may also be treated, prevented, diagnosed and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Moreover, these molecules can be used to treat, prevent, prognose, and/or diagnose anaphylaxis, hypersensitivity to an antigenic molecule, or blood group incompatibility.

Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose and/or prognose IgE-mediated allergic reactions. Such allergic reactions include, but are not limited to, asthma, rhinitis, and eczema. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to modulate IgE concentrations in vitro or in vivo.

Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention have uses in the diagnosis, prognosis, prevention, and/or treatment of inflammatory conditions. For example, since fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may inhibit the activation, proliferation and/or differentiation of cells involved in an inflammatory response, these molecules can be used to prevent and/or treat chronic and acute inflammatory conditions. Such inflammatory conditions include, but are not limited to, for example, inflammation associated with infection (e.g., septic shock, sepsis, or systemic inflammatory response syndrome), ischemia-reperfusion injury, endotoxin

lethality, complement-mediated hyperacute rejection, nephritis, cytokine or chemokine induced lung injury, inflammatory bowel disease, Crohn's disease, over production of cytokines (e.g., TNF or IL-1.), respiratory disorders (e.g., asthma and allergy); gastrointestinal disorders (e.g., inflammatory bowel disease); cancers (e.g., gastric, ovarian, lung, bladder, liver, and breast); CNS disorders (e.g., multiple sclerosis; ischemic brain injury and/or stroke, traumatic brain injury, neurodegenerative disorders (e.g., Parkinson's disease and Alzheimer's disease); AIDS-related dementia; and prion disease); cardiovascular disorders (e.g., atherosclerosis, myocarditis, cardiovascular disease, and cardiopulmonary bypass complications); as well as many additional diseases, conditions, and disorders that are characterized by inflammation (e.g., hepatitis, rheumatoid arthritis, gout, trauma, pancreatitis, sarcoidosis, dermatitis, renal ischemia-reperfusion injury, Grave's disease, systemic lupus erythematosus, diabetes mellitus, and allogenic transplant rejection).

Because inflammation is a fundamental defense mechanism, inflammatory disorders can effect virtually any tissue of the body. Accordingly, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, have uses in the treatment of tissue-specific inflammatory disorders, including, but not limited to, adrenalitis, alveolitis, angiocholecystitis, appendicitis, balanitis, blepharitis, bronchitis, bursitis, carditis, cellulitis, cervicitis, cholecystitis, chondritis, cochlitis, colitis, conjunctivitis, cystitis, dermatitis, diverticulitis, encephalitis, endocarditis, esophagitis, eustachitis, fibrositis, folliculitis, gastritis, gastroenteritis, gingivitis, glossitis, hepatosplenitis, keratitis, labyrinthitis, laryngitis, lymphangitis, mastitis, media otitis, meningitis, metritis, mucitis, myocarditis, myositis, myringitis, nephritis, neuritis, orchitis, osteochondritis, otitis, pericarditis, peritendonitis, peritonitis, pharyngitis, phlebitis, poliomyelitis, prostatitis, pulpitis, retinitis, rhinitis, salpingitis, scleritis, sclerochoroiditis, scrotitis, sinusitis, spondylitis, steatitis, stomatitis, synovitis, syringitis, tendonitis, tonsillitis, urethritis, and vaginitis.

In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, are useful to diagnose, prognose, prevent, and/or treat organ transplant rejections and graft-versus-host disease. Organ rejection occurs by host immune cell destruction of the transplanted tissue through an immune response. Similarly, an immune response is also involved in GVHD, but, in this

case, the foreign transplanted immune cells destroy the host tissues. Polypeptides, antibodies, or polynucleotides of the invention, and/or agonists or antagonists thereof, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing organ rejection or  
5 GVHD. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, that inhibit an immune response, particularly the activation, proliferation, differentiation, or chemotaxis of T-cells, may be an effective therapy in preventing experimental allergic and hyperacute xenograft rejection.

10 In other embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, are useful to diagnose, prognose, prevent, and/or treat immune complex diseases, including, but not limited to, serum sickness, post streptococcal glomerulonephritis, polyarteritis nodosa, and immune complex-induced vasculitis.

15 Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used to treat, detect, and/or prevent infectious agents. For example, by increasing the immune response, particularly increasing the proliferation activation and/or differentiation of B and/or T cells, infectious diseases may be treated, detected, and/or prevented. The immune response may be increased by either  
20 enhancing an existing immune response, or by initiating a new immune response. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also directly inhibit the infectious agent (refer to section of application listing infectious agents, etc), without necessarily eliciting an immune response.

25 In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a vaccine adjuvant that enhances immune responsiveness to an antigen. In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant to enhance tumor-specific immune  
30 responses.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant

to enhance anti-viral immune responses. Anti-viral immune responses that may be enhanced using the compositions of the invention as an adjuvant, include virus and virus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: AIDS, meningitis, Dengue, EBV, and hepatitis (e.g., hepatitis B). In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a virus, disease, or symptom selected from the group consisting of: HIV/AIDS, respiratory syncytial virus, Dengue, rotavirus, Japanese B encephalitis, influenza A and B, parainfluenza, measles, cytomegalovirus, rabies, Junin, Chikungunya, Rift Valley Fever, herpes simplex, and yellow fever.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant to enhance anti-bacterial or anti-fungal immune responses. Anti-bacterial or anti-fungal immune responses that may be enhanced using the compositions of the invention as an adjuvant, include bacteria or fungus and bacteria or fungus associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: tetanus, Diphtheria, botulism, and meningitis type B.

In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to a bacteria or fungus, disease, or symptom selected from the group consisting of: *Vibrio cholerae*, *Mycobacterium leprae*, *Salmonella typhi*, *Salmonella paratyphi*, *Meissneria meningitidis*, *Streptococcus pneumoniae*, Group B streptococcus, *Shigella spp.*, Enterotoxigenic *Escherichia coli*, Enterohemorrhagic *E. coli*, and *Borrelia burgdorferi*.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an adjuvant to enhance anti-parasitic immune responses. Anti-parasitic immune responses that may be enhanced using the compositions of the invention as an adjuvant, include parasite and parasite associated diseases or symptoms described herein or otherwise known in the art. In specific embodiments, the compositions of the invention are used as an adjuvant to



enhance an immune response to a parasite. In another specific embodiment, the compositions of the invention are used as an adjuvant to enhance an immune response to Plasmodium (malaria) or Leishmania.

5 In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be employed to treat infectious diseases including silicosis, sarcoidosis, and idiopathic pulmonary fibrosis; for example, by preventing the recruitment and activation of mononuclear phagocytes.

10 In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an antigen for the generation of antibodies to inhibit or enhance immune mediated responses against polypeptides of the invention.

15 In one embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are administered to an animal (e.g., mouse, rat, rabbit, hamster, guinea pig, pigs, micro-pig, chicken, camel, goat, horse, cow, sheep, dog, cat, non-human primate, and human, most preferably human) to boost the immune system to produce increased quantities of one or more antibodies (e.g., IgG, IgA, IgM, and IgE), to induce higher affinity antibody production and immunoglobulin class switching (e.g., IgG, IgA, IgM, and IgE), and/or to increase an  
20 immune response.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a stimulator of B cell responsiveness to pathogens.

25 In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an activator of T cells.

30 In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent that elevates the immune status of an individual prior to their receipt of immunosuppressive therapies.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to

induce higher affinity antibodies.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to increase serum immunoglobulin concentrations.

5 In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to accelerate recovery of immunocompromised individuals.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to  
10 boost immunoresponsiveness among aged populations and/or neonates.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an immune system enhancer prior to, during, or after bone marrow transplant and/or other transplants (e.g., allogeneic or xenogeneic organ transplantation). With respect to transplantation,  
15 compositions of the invention may be administered prior to, concomitant with, and/or after transplantation. In a specific embodiment, compositions of the invention are administered after transplantation, prior to the beginning of recovery of T-cell populations. In another specific embodiment, compositions of the invention are first administered after transplantation after the beginning of recovery of T cell populations, but prior to full  
20 recovery of B cell populations.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to boost immunoresponsiveness among individuals having an acquired loss of B cell function. Conditions resulting in an acquired loss of B cell function that may be  
25 ameliorated or treated by administering the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, HIV Infection, AIDS, bone marrow transplant, and B cell chronic lymphocytic leukemia (CLL).

In another specific embodiment, albumin fusion proteins of the invention and/or  
30 polynucleotides encoding albumin fusion proteins of the invention are used as an agent to boost immunoresponsiveness among individuals having a temporary immune deficiency. Conditions resulting in a temporary immune deficiency that may be ameliorated or treated

by administering the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, recovery from viral infections (e.g., influenza), conditions associated with malnutrition, recovery from infectious mononucleosis, or conditions associated with stress, recovery from measles, recovery from blood transfusion, and recovery from surgery.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a regulator of antigen presentation by monocytes, dendritic cells, and/or B-cells. In one embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention enhance antigen presentation or antagonizes antigen presentation in vitro or in vivo. Moreover, in related embodiments, this enhancement or antagonism of antigen presentation may be useful as an anti-tumor treatment or to modulate the immune system.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an agent to direct an individual's immune system towards development of a humoral response (i.e. TH2) as opposed to a TH1 cellular response.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means to induce tumor proliferation and thus make it more susceptible to anti-neoplastic agents. For example, multiple myeloma is a slowly dividing disease and is thus refractory to virtually all anti-neoplastic regimens. If these cells were forced to proliferate more rapidly their susceptibility profile would likely change.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a stimulator of B cell production in pathologies such as AIDS, chronic lymphocyte disorder and/or Common Variable Immunodeficiency.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for generation and/or regeneration of lymphoid tissues following surgery, trauma or genetic defect. In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used in the

pretreatment of bone marrow samples prior to transplant.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a gene-based therapy for genetically inherited disorders resulting in immunoincompetence/immunodeficiency such as observed among SCID patients.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means of activating monocytes/macrophages to defend against parasitic diseases that effect monocytes such as Leishmania.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means of regulating secreted cytokines that are elicited by polypeptides of the invention.

In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used in one or more of the applications described herein, as they may apply to veterinary medicine.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a means of blocking various aspects of immune responses to foreign agents or self. Examples of diseases or conditions in which blocking of certain aspects of immune responses may be desired include autoimmune disorders such as lupus, and arthritis, as well as immunoresponsiveness to skin allergies, inflammation, bowel disease, injury and diseases/disorders associated with pathogens.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for preventing the B cell proliferation and Ig secretion associated with autoimmune diseases such as idiopathic thrombocytopenic purpura, systemic lupus erythematosus and multiple sclerosis.

In another specific embodiment, polypeptides, antibodies, polynucleotides and/or agonists or antagonists of the present fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as an inhibitor of B and/or T cell migration in endothelial cells. This activity disrupts tissue architecture or cognate responses and is useful, for example in disrupting immune

responses, and blocking sepsis.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for chronic hypergammaglobulinemia evident in such diseases as monoclonal  
5 gammopathy of undetermined significance (MGUS), Waldenstrom's disease, related idiopathic monoclonal gammopathies, and plasmacytomas.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be employed for instance to inhibit polypeptide chemotaxis and activation of macrophages and their  
10 precursors, and of neutrophils, basophils, B lymphocytes and some T-cell subsets, e.g., activated and CD8 cytotoxic T cells and natural killer cells, in certain autoimmune and chronic inflammatory and infective diseases. Examples of autoimmune diseases are described herein and include multiple sclerosis, and insulin-dependent diabetes.

The albumin fusion proteins of the invention and/or polynucleotides encoding  
15 albumin fusion proteins of the invention may also be employed to treat idiopathic hyper-eosinophilic syndrome by, for example, preventing eosinophil production and migration.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to enhance or inhibit complement mediated cell lysis.

20 In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to enhance or inhibit antibody dependent cellular cytotoxicity.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be employed  
25 for treating atherosclerosis, for example, by preventing monocyte infiltration in the artery wall.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be employed to treat adult respiratory distress syndrome (ARDS).

30 In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful for stimulating wound and tissue repair, stimulating angiogenesis, and/or stimulating the

repair of vascular or lymphatic diseases or disorders. Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to stimulate the regeneration of mucosal surfaces.

In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to diagnose, prognose, treat, and/or prevent a disorder characterized by primary or acquired immunodeficiency, deficient serum immunoglobulin production, recurrent infections, and/or immune system dysfunction. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to treat or prevent infections of the joints, bones, skin, and/or parotid glands, blood-borne infections (e.g., sepsis, meningitis, septic arthritis, and/or osteomyelitis), autoimmune diseases (e.g., those disclosed herein), inflammatory disorders, and malignancies, and/or any disease or disorder or condition associated with these infections, diseases, disorders and/or malignancies) including, but not limited to, CVID, other primary immune deficiencies, HIV disease, CLL, recurrent bronchitis, sinusitis, otitis media, conjunctivitis, pneumonia, hepatitis, meningitis, herpes zoster (e.g., severe herpes zoster), and/or pneumocystis carinii. Other diseases and disorders that may be prevented, diagnosed, prognosed, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, HIV infection, HTLV-BLV infection, lymphopenia, phagocyte bactericidal dysfunction anemia, thrombocytopenia, and hemoglobinuria.

In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat, and/or diagnose an individual having common variable immunodeficiency disease ("CVID"; also known as "acquired agammaglobulinemia" and "acquired hypogammaglobulinemia") or a subset of this disease.

In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to diagnose, prognose, prevent, and/or treat cancers or neoplasms including immune cell or immune tissue-related cancers or neoplasms. Examples of cancers or neoplasms that may be prevented, diagnosed, or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not

limited to, acute myelogenous leukemia, chronic myelogenous leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, acute lymphocytic anemia (ALL) Chronic lymphocyte leukemia, plasmacytomas, multiple myeloma, Burkitt's lymphoma, EBV-transformed diseases, and/or diseases and disorders described in the section entitled  
5 "Hyperproliferative Disorders" elsewhere herein.

In another specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used as a therapy for decreasing cellular proliferation of Large B-cell Lymphomas.

In another specific embodiment, albumin fusion proteins of the invention and/or  
10 polynucleotides encoding albumin fusion proteins of the invention are used as a means of decreasing the involvement of B cells and Ig associated with Chronic Myelogenous Leukemia.

In specific embodiments, the compositions of the invention are used as an agent to boost immunoresponsiveness among B cell immunodeficient individuals, such as, for  
15 example, an individual who has undergone a partial or complete splenectomy.

#### **Blood-Related Disorders**

The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to modulate hemostatic (the stopping  
20 of bleeding) or thrombolytic (clot dissolving) activity. For example, by increasing hemostatic or thrombolytic activity, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used to treat or prevent blood coagulation diseases, disorders, and/or conditions (e.g., afibrinogenemia, factor deficiencies, hemophilia), blood platelet diseases, disorders, and/or conditions (e.g.,  
25 thrombocytopenia), or wounds resulting from trauma, surgery, or other causes. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention that can decrease hemostatic or thrombolytic activity could be used to inhibit or dissolve clotting. These molecules could be important in the treatment or prevention of heart attacks (infarction), strokes, or scarring.

In specific embodiments, the albumin fusion proteins of the invention and/or  
30 polynucleotides encoding albumin fusion proteins of the invention may be used to prevent, diagnose, prognose, and/or treat thrombosis, arterial thrombosis, venous thrombosis,

thromboembolism, pulmonary embolism, atherosclerosis, myocardial infarction, transient ischemic attack, unstable angina. In specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used for the prevention of occlusion of saphenous grafts, for reducing the risk of periprocedural thrombosis as might accompany angioplasty procedures, for reducing the risk of stroke in patients with atrial fibrillation including nonrheumatic atrial fibrillation, for reducing the risk of embolism associated with mechanical heart valves and or mitral valves disease. Other uses for the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, the prevention of occlusions in extracorporeal devices (e.g., intravascular canulas, vascular access shunts in hemodialysis patients, hemodialysis machines, and cardiopulmonary bypass machines).

In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to prevent, diagnose, prognose, and/or treat diseases and disorders of the blood and/or blood forming organs associated with the tissue(s) in which the polypeptide of the invention is expressed.

The fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to modulate hematopoietic activity (the formation of blood cells). For example, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to increase the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of anemias and leukopenias described below. Alternatively, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to decrease the quantity of all or subsets of blood cells, such as, for example, erythrocytes, lymphocytes (B or T cells), myeloid cells (e.g., basophils, eosinophils, neutrophils, mast cells, macrophages) and platelets.. The ability to decrease the quantity of blood cells or subsets of blood cells may be useful in the prevention, detection, diagnosis and/or treatment of leukocytoses, such as, for example eosinophilia.



The fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to prevent, treat, or diagnose blood dyscrasia.

Anemias are conditions in which the number of red blood cells or amount of hemoglobin (the protein that carries oxygen) in them is below normal. Anemia may be caused by excessive bleeding, decreased red blood cell production, or increased red blood cell destruction (hemolysis). The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias. Anemias that may be treated prevented or diagnosed by the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include iron deficiency anemia, hypochromic anemia, microcytic anemia, chlorosis, hereditary sideroblastic anemia, idiopathic acquired sideroblastic anemia, red cell aplasia, megaloblastic anemia (e.g., pernicious anemia, (vitamin B12 deficiency) and folic acid deficiency anemia), aplastic anemia, hemolytic anemias (e.g., autoimmune hemolytic anemia, microangiopathic hemolytic anemia, and paroxysmal nocturnal hemoglobinuria). The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias associated with diseases including but not limited to, anemias associated with systemic lupus erythematosus, cancers, lymphomas, chronic renal disease, and enlarged spleens. The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias arising from drug treatments such as anemias associated with methyldopa, dapsone, and/or sulfadruugs. Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing anemias associated with abnormal red blood cell architecture including but not limited to, hereditary spherocytosis, hereditary elliptocytosis, glucose-6-phosphate dehydrogenase deficiency, and sickle cell anemia.

The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing hemoglobin abnormalities, (e.g., those associated with sickle cell anemia, hemoglobin C disease, hemoglobin S-C disease, and hemoglobin E disease). Additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin

fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating thalassemias, including, but not limited to, major and minor forms of alpha-thalassemia and beta-thalassemia.

In another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating bleeding disorders including, but not limited to, thrombocytopenia (e.g., idiopathic thrombocytopenic purpura, and thrombotic thrombocytopenic purpura), Von Willebrand's disease, hereditary platelet disorders (e.g., storage pool disease such as Chediak-Higashi and Hermansky-Pudlak syndromes, thromboxane A2 dysfunction, thromboasthenia, and Bernard-Soulier syndrome), hemolytic-uremic syndrome, hemophelias such as hemophilia A or Factor VII deficiency and Christmas disease or Factor IX deficiency, Hereditary Hemorrhagic Telangiectasia, also known as Rendu-Osler-Weber syndrome, allergic purpura (Henoch Schonlein purpura) and disseminated intravascular coagulation.

The effect of the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention on the clotting time of blood may be monitored using any of the clotting tests known in the art including, but not limited to, whole blood partial thromboplastin time (PTT), the activated partial thromboplastin time (aPTT), the activated clotting time (ACT), the recalcified activated clotting time, or the Lee-White Clotting time.

Several diseases and a variety of drugs can cause platelet dysfunction. Thus, in a specific embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating acquired platelet dysfunction such as platelet dysfunction accompanying kidney failure, leukemia, multiple myeloma, cirrhosis of the liver, and systemic lupus erythematosus as well as platelet dysfunction associated with drug treatments, including treatment with aspirin, ticlopidine, nonsteroidal anti-inflammatory drugs (used for arthritis, pain, and sprains), and penicillin in high doses.

In another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders characterized by or associated with increased or decreased numbers of white blood cells. Leukopenia

occurs when the number of white blood cells decreases below normal. Leukopenias include, but are not limited to, neutropenia and lymphocytopenia. An increase in the number of white blood cells compared to normal is known as leukocytosis. The body generates increased numbers of white blood cells during infection. Thus, leukocytosis  
5 may simply be a normal physiological parameter that reflects infection. Alternatively, leukocytosis may be an indicator of injury or other disease such as cancer. Leukocytoses, include but are not limited to, eosinophilia, and accumulations of macrophages. In specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating leukopenia. In other specific embodiments, the  
10 albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating leukocytosis.

Leukopenia may be a generalized decreased in all types of white blood cells, or  
15 may be a specific depletion of particular types of white blood cells. Thus, in specific embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating decreases in neutrophil numbers, known as neutropenia. Neutropenias that may be diagnosed, prognosed, prevented, and/or treated by  
20 the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, infantile genetic agranulocytosis, familial neutropenia, cyclic neutropenia, neutropenias resulting from or associated with dietary deficiencies (e.g., vitamin B 12 deficiency or folic acid deficiency), neutropenias resulting from or associated with drug treatments (e.g., antibiotic regimens  
25 such as penicillin treatment, sulfonamide treatment, anticoagulant treatment, anticonvulsant drugs, anti-thyroid drugs, and cancer chemotherapy), and neutropenias resulting from increased neutrophil destruction that may occur in association with some bacterial or viral infections, allergic disorders, autoimmune diseases, conditions in which an individual has an enlarged spleen (e.g., Felty syndrome, malaria and sarcoidosis), and  
30 some drug treatment regimens.

The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing,

preventing, and/or treating lymphocytopenias (decreased numbers of B and/or T lymphocytes), including, but not limited to, lymphocytopenias resulting from or associated with stress, drug treatments (e.g., drug treatment with corticosteroids, cancer chemotherapies, and/or radiation therapies), AIDS infection and/or other diseases such as, for example, cancer, rheumatoid arthritis, systemic lupus erythematosus, chronic infections, some viral infections and/or hereditary disorders (e.g., DiGeorge syndrome, Wiskott-Aldrich Syndrome, severe combined immunodeficiency, ataxia telangiectasia).

The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with macrophage numbers and/or macrophage function including, but not limited to, Gaucher's disease, Niemann-Pick disease, Letterer-Siwe disease and Hand-Schuller-Christian disease.

In another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders associated with eosinophil numbers and/or eosinophil function including, but not limited to, idiopathic hypereosinophilic syndrome, eosinophilia-myalgia syndrome, and Hand-Schuller-Christian disease.

In yet another embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating leukemias and lymphomas including, but not limited to, acute lymphocytic (lymphoblastic) leukemia (ALL), acute myeloid (myelocytic, myelogenous, myeloblastic, or myelomonocytic) leukemia, chronic lymphocytic leukemia (e.g., B cell leukemias, T cell leukemias, Sezary syndrome, and Hairy cell leukemia), chronic myelocytic (myeloid, myelogenous, or granulocytic) leukemia, Hodgkin's lymphoma, non-hodgkin's lymphoma, Burkitt's lymphoma, and mycosis fungoides.

In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in diagnosing, prognosing, preventing, and/or treating diseases and disorders of plasma cells including, but not limited to, plasma cell dyscrasias, monoclonal gammaopathies, monoclonal gammopathies of undetermined significance, multiple myeloma,

macroglobulinemia, Waldenstrom's macroglobulinemia, cryoglobulinemia, and Raynaud's phenomenon.

5 In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating, preventing, and/or diagnosing myeloproliferative disorders, including but not limited to, polycythemia vera, relative polycythemia, secondary polycythemia, myelofibrosis, acute myelofibrosis, agnogenic myelod metaplasia, thrombocythemia, (including both primary and seconday thrombocythemia) and chronic myelocytic leukemia.

10 In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as a treatment prior to surgery, to increase blood cell production.

15 In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to enhance the migration, phagocytosis, superoxide production, antibody dependent cellular cytotoxicity of neutrophils, eosionophils and macrophages.

20 In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to increase the number of stem cells in circulation prior to stem cells pheresis. In another specific embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to increase the number of stem cells in circulation prior to platelet pheresis.

25 In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as an agent to increase cytokine production.

In other embodiments, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in preventing, diagnosing, and/or treating primary hematopoietic disorders.

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#### **Hyperproliferative Disorders**

In certain embodiments, fusion proteins of the invention and/or polynucleotides

encoding albumin fusion proteins of the invention can be used to treat or detect hyperproliferative disorders, including neoplasms. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may inhibit the proliferation of the disorder through direct or indirect interactions.

5 Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may proliferate other cells which can inhibit the hyperproliferative disorder.

For example, by increasing an immune response, particularly increasing antigenic qualities of the hyperproliferative disorder or by proliferating, differentiating, or mobilizing T-cells, hyperproliferative disorders can be treated. This immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, decreasing an immune response may also be a method of treating hyperproliferative disorders, such as a chemotherapeutic agent.

15 Examples of hyperproliferative disorders that can be treated or detected by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to neoplasms located in the: colon, abdomen, bone, breast, digestive system, liver, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be treated or detected by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Examples of such hyperproliferative disorders include, but are not limited to: Acute Childhood Lymphoblastic Leukemia, Acute Lymphoblastic Leukemia, 25 Acute Lymphocytic Leukemia, Acute Myeloid Leukemia, Adrenocortical Carcinoma, Adult (Primary) Hepatocellular Cancer, Adult (Primary) Liver Cancer, Adult Acute Lymphocytic Leukemia, Adult Acute Myeloid Leukemia, Adult Hodgkin's Disease, Adult Hodgkin's Lymphoma, Adult Lymphocytic Leukemia, Adult Non-Hodgkin's Lymphoma, Adult Primary Liver Cancer, Adult Soft Tissue Sarcoma, AIDS-Related Lymphoma, 30 AIDS-Related Malignancies, Anal Cancer, Astrocytoma, Bile Duct Cancer, Bladder Cancer, Bone Cancer, Brain Stem Glioma, Brain Tumors, Breast Cancer, Cancer of the Renal Pelvis and Ureter, Central Nervous System (Primary) Lymphoma, Central Nervous

System Lymphoma, Cerebellar Astrocytoma, Cerebral Astrocytoma, Cervical Cancer, Childhood (Primary) Hepatocellular Cancer, Childhood (Primary) Liver Cancer, Childhood Acute Lymphoblastic Leukemia, Childhood Acute Myeloid Leukemia, Childhood Brain Stem Glioma, Childhood Cerebellar Astrocytoma, Childhood Cerebral  
5 Astrocytoma, Childhood Extracranial Germ Cell Tumors, Childhood Hodgkin's Disease, Childhood Hodgkin's Lymphoma, Childhood Hypothalamic and Visual Pathway Glioma, Childhood Lymphoblastic Leukemia, Childhood Medulloblastoma, Childhood Non-Hodgkin's Lymphoma, Childhood Pineal and Supratentorial Primitive Neuroectodermal Tumors, Childhood Primary Liver Cancer, Childhood Rhabdomyosarcoma, Childhood  
10 Soft Tissue Sarcoma, Childhood Visual Pathway and Hypothalamic Glioma, Chronic Lymphocytic Leukemia, Chronic Myelogenous Leukemia, Colon Cancer, Cutaneous T-Cell Lymphoma, Endocrine Pancreas Islet Cell Carcinoma, Endometrial Cancer, Ependymoma, Epithelial Cancer, Esophageal Cancer, Ewing's Sarcoma and Related Tumors, Exocrine Pancreatic Cancer, Extracranial Germ Cell Tumor, Extragonadal Germ  
15 Cell Tumor, Extrahepatic Bile Duct Cancer, Eye Cancer, Female Breast Cancer, Gaucher's Disease, Gallbladder Cancer, Gastric Cancer, Gastrointestinal Carcinoid Tumor, Gastrointestinal Tumors, Germ Cell Tumors, Gestational Trophoblastic Tumor, Hairy Cell Leukemia, Head and Neck Cancer, Hepatocellular Cancer, Hodgkin's Disease, Hodgkin's Lymphoma, Hypergammaglobulinemia, Hypopharyngeal Cancer, Intestinal Cancers,  
20 Intraocular Melanoma, Islet Cell Carcinoma, Islet Cell Pancreatic Cancer, Kaposi's Sarcoma, Kidney Cancer, Laryngeal Cancer, Lip and Oral Cavity Cancer, Liver Cancer, Lung Cancer, Lymphoproliferative Disorders, Macroglobulinemia, Male Breast Cancer, Malignant Mesothelioma, Malignant Thymoma, Medulloblastoma, Melanoma, Mesothelioma, Metastatic Occult Primary Squamous Neck Cancer, Metastatic Primary  
25 Squamous Neck Cancer, Metastatic Squamous Neck Cancer, Multiple Myeloma, Multiple Myeloma/Plasma Cell Neoplasm, Myelodysplastic Syndrome, Myelogenous Leukemia, Myeloid Leukemia, Myeloproliferative Disorders, Nasal Cavity and Paranasal Sinus Cancer, Nasopharyngeal Cancer, Neuroblastoma, Non-Hodgkin's Lymphoma During Pregnancy, Nonmelanoma Skin Cancer, Non-Small Cell Lung Cancer, Occult Primary  
30 Metastatic Squamous Neck Cancer, Oropharyngeal Cancer, Osteo-/Malignant Fibrous Sarcoma, Osteosarcoma/Malignant Fibrous Histiocytoma, Osteosarcoma/Malignant Fibrous Histiocytoma of Bone, Ovarian Epithelial Cancer, Ovarian Germ Cell Tumor,

Ovarian Low Malignant Potential Tumor, Pancreatic Cancer, Paraproteinemias, Purpura, Parathyroid Cancer, Penile Cancer, Pheochromocytoma, Pituitary Tumor, Plasma Cell Neoplasm/Multiple Myeloma, Primary Central Nervous System Lymphoma, Primary Liver Cancer, Prostate Cancer, Rectal Cancer, Renal Cell Cancer, Renal Pelvis and Ureter Cancer, Retinoblastoma, Rhabdomyosarcoma, Salivary Gland Cancer, Sarcoidosis Sarcomas, Sezary Syndrome, Skin Cancer, Small Cell Lung Cancer, Small Intestine Cancer, Soft Tissue Sarcoma, Squamous Neck Cancer, Stomach Cancer, Supratentorial Primitive Neuroectodermal and Pineal Tumors, T-Cell Lymphoma, Testicular Cancer, Thymoma, Thyroid Cancer, Transitional Cell Cancer of the Renal Pelvis and Ureter, Transitional Renal Pelvis and Ureter Cancer, Trophoblastic Tumors, Ureter and Renal Pelvis Cell Cancer, Urethral Cancer, Uterine Cancer, Uterine Sarcoma, Vaginal Cancer, Visual Pathway and Hypothalamic Glioma, Vulvar Cancer, Waldenstrom's Macroglobulinemia, Wilms' Tumor, and any other hyperproliferative disease, besides neoplasia, located in an organ system listed above.

In another preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to diagnose, prognose, prevent, and/or treat premalignant conditions and to prevent progression to a neoplastic or malignant state, including but not limited to those disorders described above. Such uses are indicated in conditions known or suspected of preceding progression to neoplasia or cancer, in particular, where non-neoplastic cell growth consisting of hyperplasia, metaplasia, or most particularly, dysplasia has occurred (for review of such abnormal growth conditions, see Robbins and Angell, 1976, Basic Pathology, 2d Ed., W. B. Saunders Co., Philadelphia, pp. 68-79.)

Hyperplasia is a form of controlled cell proliferation, involving an increase in cell number in a tissue or organ, without significant alteration in structure or function. Hyperplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, angiofollicular mediastinal lymph node hyperplasia, angiolymphoid hyperplasia with eosinophilia, atypical melanocytic hyperplasia, basal cell hyperplasia, benign giant lymph node hyperplasia, cementum hyperplasia, congenital adrenal hyperplasia, congenital sebaceous hyperplasia, cystic hyperplasia, cystic hyperplasia of the breast, denture hyperplasia, ductal hyperplasia,



endometrial hyperplasia, fibromuscular hyperplasia, focal epithelial hyperplasia, gingival hyperplasia, inflammatory fibrous hyperplasia, inflammatory papillary hyperplasia, intravascular papillary endothelial hyperplasia, nodular hyperplasia of prostate, nodular regenerative hyperplasia, pseudoepitheliomatous hyperplasia, senile sebaceous hyperplasia, and verrucous hyperplasia.

Metaplasia is a form of controlled cell growth in which one type of adult or fully differentiated cell substitutes for another type of adult cell. Metaplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, agnogenic myeloid metaplasia, apocrine metaplasia, atypical metaplasia, autoparenchymatous metaplasia, connective tissue metaplasia, epithelial metaplasia, intestinal metaplasia, metaplastic anemia, metaplastic ossification, metaplastic polyps, myeloid metaplasia, primary myeloid metaplasia, secondary myeloid metaplasia, squamous metaplasia, squamous metaplasia of amnion, and symptomatic myeloid metaplasia.

Dysplasia is frequently a forerunner of cancer, and is found mainly in the epithelia; it is the most disorderly form of non-neoplastic cell growth, involving a loss in individual cell uniformity and in the architectural orientation of cells. Dysplastic cells often have abnormally large, deeply stained nuclei, and exhibit pleomorphism. Dysplasia characteristically occurs where there exists chronic irritation or inflammation. Dysplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, anhidrotic ectodermal dysplasia, anterofacial dysplasia, asphyxiating thoracic dysplasia, atriadigital dysplasia, bronchopulmonary dysplasia, cerebral dysplasia, cervical dysplasia, chondroectodermal dysplasia, cleidocranial dysplasia, congenital ectodermal dysplasia, craniodiaphysial dysplasia, craniocarpotarsal dysplasia, craniometaphysial dysplasia, dentin dysplasia, diaphysial dysplasia, ectodermal dysplasia, enamel dysplasia, encephalo-ophthalmic dysplasia, dysplasia epiphysialis hemimelia, dysplasia epiphysialis multiplex, dysplasia epiphysialis punctata, epithelial dysplasia, facioidigitogenital dysplasia, familial fibrous dysplasia of jaws, familial white folded dysplasia, fibromuscular dysplasia, fibrous dysplasia of bone, florid osseous dysplasia, hereditary renal-retinal dysplasia, hidrotic ectodermal dysplasia,

hypohidrotic ectodermal dysplasia, lymphopenic thymic dysplasia, mammary dysplasia, mandibulofacial dysplasia, metaphysial dysplasia, Mondini dysplasia, monostotic fibrous dysplasia, mucoepithelial dysplasia, multiple epiphysial dysplasia, oculoauriculovertebral dysplasia, oculodentodigital dysplasia, oculovertbral dysplasia, odontogenic dysplasia, ophthalmomandibulomelic dysplasia, periapical cemental dysplasia, polyostotic fibrous dysplasia, pseudoachondroplastic spondyloepiphysial dysplasia, retinal dysplasia, septo-optic dysplasia, spondyloepiphysial dysplasia, and ventriculoradial dysplasia.

Additional pre-neoplastic disorders which can be diagnosed, prognosed, prevented, and/or treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, benign dysproliferative disorders (e.g., benign tumors, fibrocystic conditions, tissue hypertrophy, intestinal polyps, colon polyps, and esophageal dysplasia), leukoplakia, keratoses, Bowen's disease, Farmer's Skin, solar cheilitis, and solar keratosis.

In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to diagnose and/or prognose disorders associated with the tissue(s) in which the polypeptide of the invention is expressed.

In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat cancers and neoplasms, including, but not limited to, those described herein. In a further preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention conjugated to a toxin or a radioactive isotope, as described herein, may be used to treat acute myelogenous leukemia.

Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may affect apoptosis, and therefore, would be useful in treating a number of diseases associated with increased cell survival or the inhibition of apoptosis. For example, diseases associated with increased cell survival or the inhibition of apoptosis that could be diagnosed, prognosed, prevented, and/or treated by polynucleotides, polypeptides, and/or agonists or antagonists of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic

cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

10 In preferred embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to inhibit growth, progression, and/or metastasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be diagnosed, prognosed, prevented, and/or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma,

astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, emangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma, and retinoblastoma.

5 Diseases associated with increased apoptosis that could be diagnosed, prognosed, prevented, and/or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, retinitis pigmentosa, cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's  
10 thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestasis (bile duct  
15 injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

Hyperproliferative diseases and/or disorders that could be diagnosed, prognosed, prevented, and/or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to,  
20 neoplasms located in the liver, abdomen, bone, breast, digestive system, pancreas, peritoneum, endocrine glands (adrenal, parathyroid, pituitary, testicles, ovary, thymus, thyroid), eye, head and neck, nervous system (central and peripheral), lymphatic system, pelvis, skin, soft tissue, spleen, thorax, and urogenital tract.

Similarly, other hyperproliferative disorders can also be diagnosed, prognosed,  
25 prevented, and/or treated by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Examples of such hyperproliferative disorders include, but are not limited to: hypergammaglobulinemia, lymphoproliferative disorders, paraproteinemias, purpura, sarcoidosis, Sezary Syndrome, Waldenström's macroglobulinemia, Gaucher's Disease, histiocytosis, and any other hyperproliferative  
30 disease, besides neoplasia, located in an organ system listed above.

Another preferred embodiment utilizes polynucleotides encoding albumin fusion proteins of the invention to inhibit aberrant cellular division, by gene therapy using the

present invention, and/or protein fusions or fragments thereof.

Thus, the present invention provides a method for treating cell proliferative disorders by inserting into an abnormally proliferating cell a polynucleotide encoding an albumin fusion protein of the present invention, wherein said polynucleotide represses said expression.

Another embodiment of the present invention provides a method of treating cell-proliferative disorders in individuals comprising administration of one or more active gene copies of the present invention to an abnormally proliferating cell or cells. In a preferred embodiment, polynucleotides of the present invention is a DNA construct comprising a recombinant expression vector effective in expressing a DNA sequence encoding said polynucleotides. In another preferred embodiment of the present invention, the DNA construct encoding the fusion protein of the present invention is inserted into cells to be treated utilizing a retrovirus, or more preferably an adenoviral vector (See G J. Nabel, et. al., PNAS 1999 96: 324-326, which is hereby incorporated by reference). In a most preferred embodiment, the viral vector is defective and will not transform non-proliferating cells, only proliferating cells. Moreover, in a preferred embodiment, the polynucleotides of the present invention inserted into proliferating cells either alone, or in combination with or fused to other polynucleotides, can then be modulated via an external stimulus (i.e. magnetic, specific small molecule, chemical, or drug administration, etc.), which acts upon the promoter upstream of said polynucleotides to induce expression of the encoded protein product. As such the beneficial therapeutic affect of the present invention may be expressly modulated (i.e. to increase, decrease, or inhibit expression of the present invention) based upon said external stimulus.

Polynucleotides of the present invention may be useful in repressing expression of oncogenic genes or antigens. By "repressing expression of the oncogenic genes " is intended the suppression of the transcription of the gene, the degradation of the gene transcript (pre-message RNA), the inhibition of splicing, the destruction of the messenger RNA, the prevention of the post-translational modifications of the protein, the destruction of the protein, or the inhibition of the normal function of the protein.

For local administration to abnormally proliferating cells, polynucleotides of the present invention may be administered by any method known to those of skill in the art including, but not limited to transfection, electroporation, microinjection of cells, or in

vehicles such as liposomes, lipofectin, or as naked polynucleotides, or any other method described throughout the specification. The polynucleotide of the present invention may be delivered by known gene delivery systems such as, but not limited to, retroviral vectors (Gilboa, J. Virology 44:845 (1982); Hocke, Nature 320:275 (1986); Wilson, et al., Proc. Natl. Acad. Sci. U.S.A. 85:3014), vaccinia virus system (Chakrabarty et al., Mol. Cell Biol. 5:3403 (1985) or other efficient DNA delivery systems (Yates et al., Nature 313:812 (1985)) known to those skilled in the art. These references are exemplary only and are hereby incorporated by reference. In order to specifically deliver or transfect cells which are abnormally proliferating and spare non-dividing cells, it is preferable to utilize a retrovirus, or adenoviral (as described in the art and elsewhere herein) delivery system known to those of skill in the art. Since host DNA replication is required for retroviral DNA to integrate and the retrovirus will be unable to self replicate due to the lack of the retrovirus genes needed for its life cycle. Utilizing such a retroviral delivery system for polynucleotides of the present invention will target said gene and constructs to abnormally proliferating cells and will spare the non-dividing normal cells.

The polynucleotides of the present invention may be delivered directly to cell proliferative disorder/disease sites in internal organs, body cavities and the like by use of imaging devices used to guide an injecting needle directly to the disease site. The polynucleotides of the present invention may also be administered to disease sites at the time of surgical intervention.

By "cell proliferative disease" is meant any human or animal disease or disorder, affecting any one or any combination of organs, cavities, or body parts, which is characterized by single or multiple local abnormal proliferations of cells, groups of cells, or tissues, whether benign or malignant.

Any amount of the polynucleotides of the present invention may be administered as long as it has a biologically inhibiting effect on the proliferation of the treated cells. Moreover, it is possible to administer more than one of the polynucleotide of the present invention simultaneously to the same site. By "biologically inhibiting" is meant partial or total growth inhibition as well as decreases in the rate of proliferation or growth of the cells. The biologically inhibitory dose may be determined by assessing the effects of the polynucleotides of the present invention on target malignant or abnormally proliferating cell growth in tissue culture, tumor growth in animals and cell cultures, or any other

method known to one of ordinary skill in the art.

Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention of the present invention are useful in inhibiting the angiogenesis of proliferative cells or tissues, either alone, as a protein fusion, or in combination with other polypeptides directly or indirectly, as described elsewhere herein. In a most preferred embodiment, said anti-angiogenesis effect may be achieved indirectly, for example, through the inhibition of hematopoietic, tumor-specific cells, such as tumor-associated macrophages (See Joseph IB, et al. J Natl Cancer Inst, 90(21):1648-53 (1998), which is hereby incorporated by reference).

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in inhibiting proliferative cells or tissues through the induction of apoptosis. These fusion proteins and/or polynucleotides may act either directly, or indirectly to induce apoptosis of proliferative cells and tissues, for example in the activation of a death-domain receptor, such as tumor necrosis factor (TNF) receptor-1, CD95 (Fas/APO-1), TNF-receptor-related apoptosis-mediated protein (TRAMP) and TNF-related apoptosis-inducing ligand (TRAIL) receptor-1 and -2 (See Schulze-Osthoff K, et.al., Eur J Biochem 254(3):439-59 (1998), which is hereby incorporated by reference). Moreover, in another preferred embodiment of the present invention, these fusion proteins and/or polynucleotides may induce apoptosis through other mechanisms, such as in the activation of other proteins which will activate apoptosis, or through stimulating the expression of these proteins, either alone or in combination with small molecule drugs or adjuvants, such as apoptonin, galectins, thioredoxins, anti-inflammatory proteins (See for example, Mutat Res 400(1-2):447-55 (1998), Med Hypotheses.50(5):423-33 (1998), Chem Biol Interact. Apr 24;111-112:23-34 (1998), J Mol Med.76(6):402-12 (1998), Int J Tissue React;20(1):3-15 (1998), which are all hereby incorporated by reference).

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are useful in inhibiting the metastasis of proliferative cells or tissues. Inhibition may occur as a direct result of administering these albumin fusion proteins and/or polynucleotides, or indirectly, such as activating the expression of proteins known to inhibit metastasis, for example alpha 4 integrins, (See, e.g., Curr Top Microbiol Immunol 1998;231:125-41, which is hereby incorporated by reference). Such thereapeutic

affects of the present invention may be achieved either alone, or in combination with small molecule drugs or adjuvants.

In another embodiment, the invention provides a method of delivering compositions containing the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention to targeted cells expressing the a polypeptide bound by, that binds to, or associates with an albumin fusion protein of the invention. Albumin fusion proteins of the invention may be associated with with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via hydrophobic, hydrophilic, ionic and/or covalent interactions.

Albumin fusion proteins of the invention are useful in enhancing the immunogenicity and/or antigenicity of proliferating cells or tissues, either directly, such as would occur if the albumin fusion proteins of the invention 'vaccinated' the immune response to respond to proliferative antigens and immunogens, or indirectly, such as in activating the expression of proteins known to enhance the immune response (e.g. chemokines), to said antigens and immunogens.

### **Renal Disorders**

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose disorders of the renal system. Renal disorders which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, kidney failure, nephritis, blood vessel disorders of kidney, metabolic and congenital kidney disorders, urinary disorders of the kidney, autoimmune disorders, sclerosis and necrosis, electrolyte imbalance, and kidney cancers.

Kidney diseases which can be diagnosed, prognosed, prevented, and/or treated with compositions of the invention include, but are not limited to, acute kidney failure, chronic kidney failure, atheroembolic renal failure, end-stage renal disease, inflammatory diseases of the kidney (e.g., acute glomerulonephritis, postinfectious glomerulonephritis, rapidly progressive glomerulonephritis, nephrotic syndrome, membranous glomerulonephritis, familial nephrotic syndrome, membranoproliferative glomerulonephritis I and II, mesangial proliferative glomerulonephritis, chronic glomerulonephritis, acute



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tubulointerstitial nephritis, chronic tubulointerstitial nephritis, acute post-streptococcal glomerulonephritis (PSGN), pyelonephritis, lupus nephritis, chronic nephritis, interstitial nephritis, and post-streptococcal glomerulonephritis), blood vessel disorders of the kidneys (e.g., kidney infarction, atheroembolic kidney disease, cortical necrosis, malignant nephrosclerosis, renal vein thrombosis, renal underperfusion, renal retinopathy, renal ischemia-reperfusion, renal artery embolism, and renal artery stenosis), and kidney disorders resulting from urinary tract disease (e.g., pyelonephritis, hydronephrosis, urolithiasis (renal lithiasis, nephrolithiasis), reflux nephropathy, urinary tract infections, urinary retention, and acute or chronic unilateral obstructive uropathy.)

10 In addition, compositions of the invention can be used to diagnose, prognose, prevent, and/or treat metabolic and congenital disorders of the kidney (e.g., uremia, renal amyloidosis, renal osteodystrophy, renal tubular acidosis, renal glycosuria, nephrogenic diabetes insipidus, cystinuria, Fanconi's syndrome, renal fibrocystic osteosis (renal rickets), Hartnup disease, Bartter's syndrome, Liddle's syndrome, polycystic kidney disease, medullary cystic disease, medullary sponge kidney, Alport's syndrome, nail-patella syndrome, congenital nephrotic syndrome, CRUSH syndrome, horseshoe kidney, diabetic nephropathy, nephrogenic diabetes insipidus, analgesic nephropathy, kidney stones, and membranous nephropathy), and autoimmune disorders of the kidney (e.g., systemic lupus erythematosus (SLE), Goodpasture syndrome, IgA nephropathy, and IgM mesangial proliferative glomerulonephritis).

20 Compositions of the invention can also be used to diagnose, prognose, prevent, and/or treat sclerotic or necrotic disorders of the kidney (e.g., glomerulosclerosis, diabetic nephropathy, focal segmental glomerulosclerosis (FSGS), necrotizing glomerulonephritis, and renal papillary necrosis), cancers of the kidney (e.g., nephroma, hypernephroma, nephroblastoma, renal cell cancer, transitional cell cancer, renal adenocarcinoma, squamous cell cancer, and Wilm's tumor), and electrolyte imbalances (e.g., nephrocalcinosis, pyuria, edema, hydronephritis, proteinuria, hyponatremia, hypernatremia, hypokalemia, hyperkalemia, hypocalcemia, hypercalcemia, hypophosphatemia, and hyperphosphatemia).

30 Compositions of the invention may be administered using any method known in the art, including, but not limited to, direct needle injection at the delivery site, intravenous injection, topical administration, catheter infusion, biolistic injectors, particle accelerators,

gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Compositions of the invention may be administered as part of a Therapeutic, described in more detail below.

5 Methods of delivering polynucleotides of the invention are described in more detail herein.

### **Cardiovascular Disorders**

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose cardiovascular disorders, including, but not limited to, peripheral artery disease, such as limb ischemia.

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Cardiovascular disorders include, but are not limited to, cardiovascular abnormalities, such as arterio-arterial fistula, arteriovenous fistula, cerebral arteriovenous malformations, congenital heart defects, pulmonary atresia, and Scimitar Syndrome. Congenital heart defects include, but are not limited to, aortic coarctation, cor triatriatum, coronary vessel anomalies, crisscross heart, dextrocardia, patent ductus arteriosus, Ebstein's anomaly, Eisenmenger complex, hypoplastic left heart syndrome, levocardia, tetralogy of fallot, transposition of great vessels, double outlet right ventricle, tricuspid atresia, persistent truncus arteriosus, and heart septal defects, such as aortopulmonary septal defect, endocardial cushion defects, Lutembacher's Syndrome, trilogy of Fallot, ventricular heart septal defects.

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Cardiovascular disorders also include, but are not limited to, heart disease, such as arrhythmias, carcinoid heart disease, high cardiac output, low cardiac output, cardiac tamponade, endocarditis (including bacterial), heart aneurysm, cardiac arrest, congestive heart failure, congestive cardiomyopathy, paroxysmal dyspnea, cardiac edema, heart hypertrophy, congestive cardiomyopathy, left ventricular hypertrophy, right ventricular hypertrophy, post-infarction heart rupture, ventricular septal rupture, heart valve diseases, myocardial diseases, myocardial ischemia, pericardial effusion, pericarditis (including constrictive and tuberculous), pneumopericardium, postpericardiotomy syndrome, pulmonary heart disease, rheumatic heart disease, ventricular dysfunction, hyperemia, cardiovascular pregnancy complications, Scimitar Syndrome, cardiovascular syphilis, and

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cardiovascular tuberculosis.

Arrhythmias include, but are not limited to, sinus arrhythmia, atrial fibrillation, atrial flutter, bradycardia, extrasystole, Adams-Stokes Syndrome, bundle-branch block, sinoatrial block, long QT syndrome, parasystole, Lown-Ganong-Levine Syndrome, 5 Mahaim-type pre-excitation syndrome, Wolff-Parkinson-White syndrome, sick sinus syndrome, tachycardias, and ventricular fibrillation. Tachycardias include paroxysmal tachycardia, supraventricular tachycardia, accelerated idioventricular rhythm, atrioventricular nodal reentry tachycardia, ectopic atrial tachycardia, ectopic junctional tachycardia, sinoatrial nodal reentry tachycardia, sinus tachycardia, Torsades de Pointes, 10 and ventricular tachycardia.

Heart valve diseases include, but are not limited to, aortic valve insufficiency, aortic valve stenosis, hear murmurs, aortic valve prolapse, mitral valve prolapse, tricuspid valve prolapse, mitral valve insufficiency, mitral valve stenosis, pulmonary atresia, pulmonary valve insufficiency, pulmonary valve stenosis, tricuspid atresia, tricuspid valve 15 insufficiency, and tricuspid valve stenosis.

Myocardial diseases include, but are not limited to, alcoholic cardiomyopathy, congestive cardiomyopathy, hypertrophic cardiomyopathy, aortic subvalvular stenosis, pulmonary subvalvular stenosis, restrictive cardiomyopathy, Chagas cardiomyopathy, endocardial fibroelastosis, endomyocardial fibrosis, Kearns Syndrome, myocardial 20 reperfusion injury, and myocarditis.

Myocardial ischemias include, but are not limited to, coronary disease, such as angina pectoris, coronary aneurysm, coronary arteriosclerosis, coronary thrombosis, coronary vasospasm, myocardial infarction and myocardial stunning.

Cardiovascular diseases also include vascular diseases such as aneurysms, 25 angiodyplasia, angiomatosis, bacillary angiomatosis, Hippel-Lindau Disease, Klippel-Trenaunay-Weber Syndrome, Sturge-Weber Syndrome, angioneurotic edema, aortic diseases, Takayasu's Arteritis, aortitis, Leriche's Syndrome, arterial occlusive diseases, arteritis, enarteritis, polyarteritis nodosa, cerebrovascular disorders, diabetic angiopathies, diabetic retinopathy, embolisms, thrombosis, erythromelalgia, hemorrhoids, hepatic veno- 30 occlusive disease, hypertension, hypotension, ischemia, peripheral vascular diseases, phlebitis, pulmonary veno-occlusive disease, Raynaud's disease, CREST syndrome, retinal vein occlusion, Scimitar syndrome, superior vena cava syndrome, telangiectasia, atacia



gelfoam sponge depots, other commercially available depot materials, osmotic pumps, oral or suppository solid pharmaceutical formulations, decanting or topical applications during surgery, aerosol delivery. Such methods are known in the art. Methods of delivering polynucleotides are described in more detail herein.

5

### **Respiratory Disorders**

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to treat, prevent, diagnose, and/or prognose diseases and/or disorders of the respiratory system.

Diseases and disorders of the respiratory system include, but are not limited to, nasal vestibulitis, nonallergic rhinitis (e.g., acute rhinitis, chronic rhinitis, atrophic rhinitis, vasomotor rhinitis), nasal polyps, and sinusitis; juvenile angiofibromas, cancer of the nose and juvenile papillomas, vocal cord polyps, nodules (singer's nodules), contact ulcers, vocal cord paralysis, laryngoceles, pharyngitis (e.g., viral and bacterial), tonsillitis, tonsillar cellulitis, parapharyngeal abscess, laryngitis, laryngoceles, and throat cancers (e.g., cancer of the nasopharynx, tonsil cancer, larynx cancer), lung cancer (e.g., squamous cell carcinoma, small cell (oat cell) carcinoma, large cell carcinoma, and adenocarcinoma), allergic disorders (eosinophilic pneumonia, hypersensitivity pneumonitis (e.g., extrinsic allergic alveolitis, allergic interstitial pneumonitis, organic dust pneumoconiosis, allergic bronchopulmonary aspergillosis, asthma, Wegener's granulomatosis (granulomatous vasculitis), Goodpasture's syndrome)), pneumonia (e.g., bacterial pneumonia (e.g., *Streptococcus pneumoniae* (pneumococcal pneumonia), *Staphylococcus aureus* (staphylococcal pneumonia), Gram-negative bacterial pneumonia (caused by, e.g., *Klebsiella* and *Pseudomonas spp.*), *Mycoplasma pneumoniae* pneumonia, *Hemophilus influenzae* pneumonia, *Legionella pneumophila* (Legionnaires' disease), and *Chlamydia psittaci* (Psittacosis)), and viral pneumonia (e.g., influenza, chickenpox (varicella).

Additional diseases and disorders of the respiratory system include, but are not limited to bronchiolitis, polio (poliomyelitis), croup, respiratory syncytial viral infection, mumps, erythema infectiosum (fifth disease), roseola infantum, progressive rubella panencephalitis, german measles, and subacute sclerosing panencephalitis), fungal pneumonia (e.g., Histoplasmosis, Coccidioidomycosis, Blastomycosis, fungal infections in

people with severely suppressed immune systems (e.g., cryptococcosis, caused by *Cryptococcus neoformans*; aspergillosis, caused by *Aspergillus spp.*; candidiasis, caused by *Candida*; and mucormycosis)), *Pneumocystis carinii* (pneumocystis pneumonia), atypical pneumonias (e.g., *Mycoplasma* and *Chlamydia spp.*), opportunistic infection pneumonia, nosocomial pneumonia, chemical pneumonitis, and aspiration pneumonia, pleural disorders (e.g., pleurisy, pleural effusion, and pneumothorax (e.g., simple spontaneous pneumothorax, complicated spontaneous pneumothorax, tension pneumothorax)), obstructive airway diseases (e.g., asthma, chronic obstructive pulmonary disease (COPD), emphysema, chronic or acute bronchitis), occupational lung diseases (e.g., silicosis, black lung (coal workers' pneumoconiosis), asbestosis, berylliosis, occupational asthma, byssinosis, and benign pneumoconioses), Infiltrative Lung Disease (e.g., pulmonary fibrosis (e.g., fibrosing alveolitis, usual interstitial pneumonia), idiopathic pulmonary fibrosis, desquamative interstitial pneumonia, lymphoid interstitial pneumonia, histiocytosis X (e.g., Letterer-Siwe disease, Hand-Schüller-Christian disease, eosinophilic granuloma), idiopathic pulmonary hemosiderosis, sarcoidosis and pulmonary alveolar proteinosis), Acute respiratory distress syndrome (also called, e.g., adult respiratory distress syndrome), edema, pulmonary embolism, bronchitis (e.g., viral, bacterial), bronchiectasis, atelectasis, lung abscess (caused by, e.g., *Staphylococcus aureus* or *Legionella pneumophila*), and cystic fibrosis.

### Anti-Angiogenesis Activity

The naturally occurring balance between endogenous stimulators and inhibitors of angiogenesis is one in which inhibitory influences predominate. Rastinejad *et al.*, *Cell* 56:345-355 (1989). In those rare instances in which neovascularization occurs under normal physiological conditions, such as wound healing, organ regeneration, embryonic development, and female reproductive processes, angiogenesis is stringently regulated and spatially and temporally delimited. Under conditions of pathological angiogenesis such as that characterizing solid tumor growth, these regulatory controls fail. Unregulated angiogenesis becomes pathologic and sustains progression of many neoplastic and non-neoplastic diseases. A number of serious diseases are dominated by abnormal neovascularization including solid tumor growth and metastases, arthritis, some types of

eye disorders, and psoriasis. See, e.g., reviews by Moses *et al.*, *Biotech.* 9:630-634 (1991); Folkman *et al.*, *N. Engl. J. Med.*, 333:1757-1763 (1995); Auerbach *et al.*, *J. Microvasc. Res.* 29:401-411 (1985); Folkman, *Advances in Cancer Research*, eds. Klein and Weinhouse, Academic Press, New York, pp. 175-203 (1985); Patz, *Am. J. Ophthalmol.* 94:715-743 (1982); and Folkman *et al.*, *Science* 221:719-725 (1983). In a number of pathological conditions, the process of angiogenesis contributes to the disease state. For example, significant data have accumulated which suggest that the growth of solid tumors is dependent on angiogenesis. Folkman and Klagsbrun, *Science* 235:442-447 (1987).

The present invention provides for treatment of diseases or disorders associated with neovascularization by administration of fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Malignant and metastatic conditions which can be treated with the polynucleotides and polypeptides, or agonists or antagonists of the invention include, but are not limited to, malignancies, solid tumors, and cancers described herein and otherwise known in the art (for a review of such disorders, see Fishman *et al.*, *Medicine*, 2d Ed., J. B. Lippincott Co., Philadelphia (1985)). Thus, the present invention provides a method of treating an angiogenesis-related disease and/or disorder, comprising administering to an individual in need thereof a therapeutically effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention. For example, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be utilized in a variety of additional methods in order to therapeutically treat a cancer or tumor. Cancers which may be treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to solid tumors, including prostate, lung, breast, ovarian, stomach, pancreas, larynx, esophagus, testes, liver, parotid, biliary tract, colon, rectum, cervix, uterus, endometrium, kidney, bladder, thyroid cancer; primary tumors and metastases; melanomas; glioblastoma; Kaposi's sarcoma; leiomyosarcoma; non-small cell lung cancer; colorectal cancer; advanced malignancies; and blood born tumors such as leukemias. For example, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be delivered topically, in order to treat cancers such as skin cancer, head and neck tumors, breast tumors, and Kaposi's sarcoma.

Within yet other aspects, fusion proteins of the invention and/or polynucleotides

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encoding albumin fusion proteins of the invention may be utilized to treat superficial forms of bladder cancer by, for example, intravesical administration. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be delivered directly into the tumor, or near the tumor site, via injection or a catheter. Of course, as the artisan of ordinary skill will appreciate, the appropriate mode of administration will vary according to the cancer to be treated. Other modes of delivery are discussed herein.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful in treating other disorders, besides cancers, which involve angiogenesis. These disorders include, but are not limited to: benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis.

For example, within one aspect of the present invention methods are provided for treating hypertrophic scars and keloids, comprising the step of administering albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention to a hypertrophic scar or keloid.

Within one embodiment of the present invention fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are directly injected into a hypertrophic scar or keloid, in order to prevent the progression of these lesions. This therapy is of particular value in the prophylactic treatment of conditions which are known to result in the development of hypertrophic scars and keloids (e.g., burns), and is preferably initiated after the proliferative phase has had time to progress (approximately 14 days after the initial injury), but before hypertrophic scar or keloid



development. As noted above, the present invention also provides methods for treating neovascular diseases of the eye, including for example, corneal neovascularization, neovascular glaucoma, proliferative diabetic retinopathy, retrolental fibroplasia and macular degeneration.

5           Moreover, Ocular disorders associated with neovascularization which can be treated with the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to: neovascular glaucoma, diabetic retinopathy, retinoblastoma, retrolental fibroplasia, uveitis, retinopathy of prematurity macular degeneration, corneal graft neovascularization, as well as other eye  
10 inflammatory diseases, ocular tumors and diseases associated with choroidal or iris neovascularization. See, e.g., reviews by Waltman *et al.*, *Am. J. Ophthalmol.* 85:704-710 (1978) and Gartner *et al.*, *Surv. Ophthalmol.* 22:291-312 (1978).

          Thus, within one aspect of the present invention methods are provided for treating neovascular diseases of the eye such as corneal neovascularization (including corneal graft  
15 neovascularization), comprising the step of administering to a patient a therapeutically effective amount of a compound (e.g., fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention) to the cornea, such that the formation of blood vessels is inhibited. Briefly, the cornea is a tissue which normally lacks blood vessels. In certain pathological conditions however, capillaries may extend  
20 into the cornea from the pericorneal vascular plexus of the limbus. When the cornea becomes vascularized, it also becomes clouded, resulting in a decline in the patient's visual acuity. Visual loss may become complete if the cornea completely opacitates. A wide variety of disorders can result in corneal neovascularization, including for example, corneal infections (e.g., trachoma, herpes simplex keratitis, leishmaniasis and  
25 onchocerciasis), immunological processes (e.g., graft rejection and Stevens-Johnson's syndrome), alkali burns, trauma, inflammation (of any cause), toxic and nutritional deficiency states, and as a complication of wearing contact lenses.

          Within particularly preferred embodiments of the invention, may be prepared for topical administration in saline (combined with any of the preservatives and antimicrobial  
30 agents commonly used in ocular preparations), and administered in eyedrop form. The solution or suspension may be prepared in its pure form and administered several times daily. Alternatively, anti-angiogenic compositions, prepared as described above, may also

be administered directly to the cornea. Within preferred embodiments, the anti-angiogenic composition is prepared with a muco-adhesive polymer which binds to cornea. Within further embodiments, the anti-angiogenic factors or anti-angiogenic compositions may be utilized as an adjunct to conventional steroid therapy. Topical therapy may also be useful prophylactically in corneal lesions which are known to have a high probability of inducing an angiogenic response (such as chemical burns). In these instances the treatment, likely in combination with steroids, may be instituted immediately to help prevent subsequent complications.

Within other embodiments, the compounds described above may be injected directly into the corneal stroma by an ophthalmologist under microscopic guidance. The preferred site of injection may vary with the morphology of the individual lesion, but the goal of the administration would be to place the composition at the advancing front of the vasculature (i.e., interspersed between the blood vessels and the normal cornea). In most cases this would involve perilimbic corneal injection to "protect" the cornea from the advancing blood vessels. This method may also be utilized shortly after a corneal insult in order to prophylactically prevent corneal neovascularization. In this situation the material could be injected in the perilimbic cornea interspersed between the corneal lesion and its undesired potential limbic blood supply. Such methods may also be utilized in a similar fashion to prevent capillary invasion of transplanted corneas. In a sustained-release form injections might only be required 2-3 times per year. A steroid could also be added to the injection solution to reduce inflammation resulting from the injection itself.

Within another aspect of the present invention, methods are provided for treating neovascular glaucoma, comprising the step of administering to a patient a therapeutically effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention to the eye, such that the formation of blood vessels is inhibited. In one embodiment, the compound may be administered topically to the eye in order to treat early forms of neovascular glaucoma. Within other embodiments, the compound may be implanted by injection into the region of the anterior chamber angle. Within other embodiments, the compound may also be placed in any location such that the compound is continuously released into the aqueous humor. Within another aspect of the present invention, methods are provided for treating proliferative diabetic retinopathy, comprising the step of administering to a patient a therapeutically

effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention to the eyes, such that the formation of blood vessels is inhibited.

5        Within particularly preferred embodiments of the invention, proliferative diabetic retinopathy may be treated by injection into the aqueous humor or the vitreous, in order to increase the local concentration of the polynucleotide, polypeptide, antagonist and/or agonist in the retina. Preferably, this treatment should be initiated prior to the acquisition of severe disease requiring photocoagulation.

10        Within another aspect of the present invention, methods are provided for treating retrolental fibroplasia, comprising the step of administering to a patient a therapeutically effective amount of an albumin fusion protein of the invention and/or polynucleotides encoding an albumin fusion protein of the invention to the eye, such that the formation of blood vessels is inhibited. The compound may be administered topically, via intravitreal injection and/or via intraocular implants.

15        Additionally, disorders which can be treated with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, hemangioma, arthritis, psoriasis, angiofibroma, atherosclerotic plaques, delayed wound healing, granulations, hemophilic joints, hypertrophic scars, nonunion fractures, Osler-Weber syndrome, pyogenic granuloma, scleroderma, trachoma, and  
20        vascular adhesions.

         Moreover, disorders and/or states, which can be treated, prevented, diagnosed, and/or prognosed with the the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention of the invention include, but are not limited to, solid tumors, blood born tumors such as leukemias, tumor  
25        metastasis, Kaposi's sarcoma, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas, rheumatoid arthritis, psoriasis, ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, and uveitis, delayed wound healing,  
30        endometriosis, vasculogenesis, granulations, hypertrophic scars (keloids), nonunion fractures, scleroderma, trachoma, vascular adhesions, myocardial angiogenesis, coronary collaterals, cerebral collaterals, arteriovenous malformations, ischemic limb angiogenesis,

Osler-Webber Syndrome, plaque neovascularization, telangiectasia, hemophiliac joints, angiofibroma, fibromuscular dysplasia, wound granulation, Crohn's disease, atherosclerosis, birth control agent by preventing vascularization required for embryo implantation controlling menstruation, diseases that have angiogenesis as a pathologic consequence such as cat scratch disease (*Rochela minalia quintosa*), ulcers (*Helicobacter pylori*), Bartonellosis and bacillary angiomatosis.

In one aspect of the birth control method, an amount of the compound sufficient to block embryo implantation is administered before or after intercourse and fertilization have occurred, thus providing an effective method of birth control, possibly a "morning after" method. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be used in controlling menstruation or administered as either a peritoneal lavage fluid or for peritoneal implantation in the treatment of endometriosis.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be incorporated into surgical sutures in order to prevent stitch granulomas.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be utilized in a wide variety of surgical procedures. For example, within one aspect of the present invention a compositions (in the form of, for example, a spray or film) may be utilized to coat or spray an area prior to removal of a tumor, in order to isolate normal surrounding tissues from malignant tissue, and/or to prevent the spread of disease to surrounding tissues. Within other aspects of the present invention, compositions (e.g., in the form of a spray) may be delivered via endoscopic procedures in order to coat tumors, or inhibit angiogenesis in a desired locale. Within yet other aspects of the present invention, surgical meshes which have been coated with anti-angiogenic compositions of the present invention may be utilized in any procedure wherein a surgical mesh might be utilized. For example, within one embodiment of the invention a surgical mesh laden with an anti-angiogenic composition may be utilized during abdominal cancer resection surgery (e.g., subsequent to colon resection) in order to provide support to the structure, and to release an amount of the anti-angiogenic factor.

Within further aspects of the present invention, methods are provided for treating tumor excision sites, comprising administering albumin fusion proteins of the invention

and/or polynucleotides encoding albumin fusion proteins of the invention to the resection margins of a tumor subsequent to excision, such that the local recurrence of cancer and the formation of new blood vessels at the site is inhibited. Within one embodiment of the invention, the anti-angiogenic compound is administered directly to the tumor excision site (e.g., applied by swabbing, brushing or otherwise coating the resection margins of the tumor with the anti-angiogenic compound). Alternatively, the anti-angiogenic compounds may be incorporated into known surgical pastes prior to administration. Within particularly preferred embodiments of the invention, the anti-angiogenic compounds are applied after hepatic resections for malignancy, and after neurosurgical operations.

Within one aspect of the present invention, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be administered to the resection margin of a wide variety of tumors, including for example, breast, colon, brain and hepatic tumors. For example, within one embodiment of the invention, anti-angiogenic compounds may be administered to the site of a neurological tumor subsequent to excision, such that the formation of new blood vessels at the site are inhibited.

The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be administered along with other anti-angiogenic factors. Representative examples of other anti-angiogenic factors include: Anti-Invasive Factor, retinoic acid and derivatives thereof, paclitaxel, Suramin, Tissue Inhibitor of Metalloproteinase-1, Tissue Inhibitor of Metalloproteinase-2, Plasminogen Activator Inhibitor-1, Plasminogen Activator Inhibitor-2, and various forms of the lighter "d group" transition metals.

Lighter "d group" transition metals include, for example, vanadium, molybdenum, tungsten, titanium, niobium, and tantalum species. Such transition metal species may form transition metal complexes. Suitable complexes of the above-mentioned transition metal species include oxo transition metal complexes.

Representative examples of vanadium complexes include oxo vanadium complexes such as vanadate and vanadyl complexes. Suitable vanadate complexes include metavanadate and orthovanadate complexes such as, for example, ammonium metavanadate, sodium metavanadate, and sodium orthovanadate. Suitable vanadyl complexes include, for example, vanadyl acetylacetonate and vanadyl sulfate including vanadyl sulfate hydrates such as vanadyl sulfate mono- and trihydrates.

Representative examples of tungsten and molybdenum complexes also include oxo complexes. Suitable oxo tungsten complexes include tungstate and tungsten oxide complexes. Suitable tungstate complexes include ammonium tungstate, calcium tungstate, sodium tungstate dihydrate, and tungstic acid. Suitable tungsten oxides include tungsten (IV) oxide and tungsten (VI) oxide. Suitable oxo molybdenum complexes include molybdate, molybdenum oxide, and molybdenyl complexes. Suitable molybdate complexes include ammonium molybdate and its hydrates, sodium molybdate and its hydrates, and potassium molybdate and its hydrates. Suitable molybdenum oxides include molybdenum (VI) oxide, molybdenum (VI) oxide, and molybdic acid. Suitable molybdenyl complexes include, for example, molybdenyl acetylacetonate. Other suitable tungsten and molybdenum complexes include hydroxo derivatives derived from, for example, glycerol, tartaric acid, and sugars.

A wide variety of other anti-angiogenic factors may also be utilized within the context of the present invention. Representative examples include platelet factor 4; protamine sulphate; sulphated chitin derivatives (prepared from queen crab shells), (Murata et al., Cancer Res. 51:22-26, 1991); Sulphated Polysaccharide Peptidoglycan Complex (SP- PG) (the function of this compound may be enhanced by the presence of steroids such as estrogen, and tamoxifen citrate); Staurosporine; modulators of matrix metabolism, including for example, proline analogs, cishydroxyproline, d,L-3,4-dehydroproline, Thiaproline, alpha,alpha-dipyridyl, aminopropionitrile fumarate; 4-propyl-5-(4-pyridinyl)-2(3H)-oxazolone; Methotrexate; Mitoxantrone; Heparin; Interferons; 2 Macroglobulin-serum; ChIMP-3 (Pavloff et al., J. Bio. Chem. 267:17321-17326, (1992)); Chymostatin (Tomkinson et al., Biochem J. 286:475-480, (1992)); Cyclodextrin Tetradeccasulfate; Eponemycin; Camptothecin; Fumagillin (Ingber et al., Nature 348:555-557, 1990); Gold Sodium Thiomalate ("GST"; Matsubara and Ziff, J. Clin. Invest. 79:1440-1446, (1987)); anticollagenase-serum; alpha2-antiplasmin (Holmes et al., J. Biol. Chem. 262(4):1659-1664, (1987)); Bisantrene (National Cancer Institute); Lobenzarit disodium (N-(2)-carboxyphenyl-4- chloroanthronilic acid disodium or "CCA"; Takeuchi et al., Agents Actions 36:312-316, (1992)); Thalidomide; Angostatic steroid; AGM-1470; carboxyaminoimidazole; and metalloproteinase inhibitors such as BB94.

### Diseases at the Cellular Level

Diseases associated with increased cell survival or the inhibition of apoptosis that could be treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include cancers (such as follicular lymphomas, carcinomas with p53 mutations, and hormone-dependent tumors, including, but not limited to colon cancer, cardiac tumors, pancreatic cancer, melanoma, retinoblastoma, glioblastoma, lung cancer, intestinal cancer, testicular cancer, stomach cancer, neuroblastoma, myxoma, myoma, lymphoma, endothelioma, osteoblastoma, osteoclastoma, osteosarcoma, chondrosarcoma, adenoma, breast cancer, prostate cancer, Kaposi's sarcoma and ovarian cancer); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) and viral infections (such as herpes viruses, pox viruses and adenoviruses), inflammation, graft v. host disease, acute graft rejection, and chronic graft rejection.

In preferred embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to inhibit growth, progression, and/or metasis of cancers, in particular those listed above.

Additional diseases or conditions associated with increased cell survival that could be treated or detected by fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (e.g., acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (e.g., chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (e.g., Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyosarcoma, colon carcinoma, pancreatic cancer, breast cancer,

ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma, and retinoblastoma.

Diseases associated with increased apoptosis that could be treated, prevented, diagnosed, and/or prognosed using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, include, but are not limited to, AIDS; neurodegenerative disorders (such as Alzheimer's disease, Parkinson's disease, Amyotrophic lateral sclerosis, Retinitis pigmentosa, Cerebellar degeneration and brain tumor or prior associated disease); autoimmune disorders (such as, multiple sclerosis, Sjogren's syndrome, Hashimoto's thyroiditis, biliary cirrhosis, Behcet's disease, Crohn's disease, polymyositis, systemic lupus erythematosus and immune-related glomerulonephritis and rheumatoid arthritis) myelodysplastic syndromes (such as aplastic anemia), graft v. host disease, ischemic injury (such as that caused by myocardial infarction, stroke and reperfusion injury), liver injury (e.g., hepatitis related liver injury, ischemia/reperfusion injury, cholestosis (bile duct injury) and liver cancer); toxin-induced liver disease (such as that caused by alcohol), septic shock, cachexia and anorexia.

#### **Wound Healing and Epithelial Cell Proliferation**

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, for therapeutic purposes, for example, to stimulate epithelial cell proliferation and basal keratinocytes for the purpose of wound healing, and to stimulate hair follicle production and healing of dermal wounds. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be clinically useful in stimulating wound healing including surgical wounds, excisional wounds, deep wounds involving damage of the dermis and epidermis,



eye tissue wounds, dental tissue wounds, oral cavity wounds, diabetic ulcers, dermal ulcers, cubitus ulcers, arterial ulcers, venous stasis ulcers, burns resulting from heat exposure or chemicals, and other abnormal wound healing conditions such as uremia, malnutrition, vitamin deficiencies and complications associated with systemic treatment  
5 with steroids, radiation therapy and antineoplastic drugs and antimetabolites. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to promote dermal reestablishment subsequent to dermal loss

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin  
10 fusion proteins of the invention, could be used to increase the adherence of skin grafts to a wound bed and to stimulate re-epithelialization from the wound bed. The following are types of grafts that fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to increase adherence to a wound bed: autografts, artificial skin, allografts, autodermic graft, autoepdermic grafts, avacular  
15 grafts, Blair-Brown grafts, bone graft, brephoplastic grafts, cutis graft, delayed graft, dermic graft, epidermic graft, fascia graft, full thickness graft, heterologous graft, xenograft, homologous graft, hyperplastic graft, lamellar graft, mesh graft, mucosal graft, Ollier-Thiersch graft, omenpal graft, patch graft, pedicle graft, penetrating graft, split skin graft, thick split graft. Albumin fusion proteins of the invention and/or polynucleotides  
20 encoding albumin fusion proteins of the invention, can be used to promote skin strength and to improve the appearance of aged skin.

It is believed that fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, will also produce changes in hepatocyte proliferation, and epithelial cell proliferation in the lung, breast, pancreas, stomach, small  
25 intestine, and large intestine. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could promote proliferation of epithelial cells such as sebocytes, hair follicles, hepatocytes, type II pneumocytes, mucin-producing goblet cells, and other epithelial cells and their progenitors contained within the skin, lung, liver, and gastrointestinal tract. Albumin fusion proteins of  
30 the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may promote proliferation of endothelial cells, keratinocytes, and basal keratinocytes.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin

fusion proteins of the invention, could also be used to reduce the side effects of gut toxicity that result from radiation, chemotherapy treatments or viral infections. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may have a cytoprotective effect on the small intestine mucosa. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may also stimulate healing of mucositis (mouth ulcers) that result from chemotherapy and viral infections.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could further be used in full regeneration of skin in full and partial thickness skin defects, including burns, (i.e., repopulation of hair follicles, sweat glands, and sebaceous glands), treatment of other skin defects such as psoriasis. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to treat epidermolysis bullosa, a defect in adherence of the epidermis to the underlying dermis which results in frequent, open and painful blisters by accelerating reepithelialization of these lesions. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could also be used to treat gastric and duodenal ulcers and help heal by scar formation of the mucosal lining and regeneration of glandular mucosa and duodenal mucosal lining more rapidly. Inflammatory bowel diseases, such as Crohn's disease and ulcerative colitis, are diseases which result in destruction of the mucosal surface of the small or large intestine, respectively. Thus, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to promote the resurfacing of the mucosal surface to aid more rapid healing and to prevent progression of inflammatory bowel disease. Treatment with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, is expected to have a significant effect on the production of mucus throughout the gastrointestinal tract and could be used to protect the intestinal mucosa from injurious substances that are ingested or following surgery. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to treat diseases associated with the under expression.

Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to prevent and heal damage to the

lungs due to various pathological states. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, which could stimulate proliferation and differentiation and promote the repair of alveoli and bronchiolar epithelium to prevent or treat acute or chronic lung damage. For example, emphysema, which results in the progressive loss of alveoli, and inhalation injuries, i.e., resulting from smoke inhalation and burns, that cause necrosis of the bronchiolar epithelium and alveoli could be effectively treated using polynucleotides or polypeptides, agonists or antagonists of the present invention. Also fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to stimulate the proliferation of and differentiation of type II pneumocytes, which may help treat or prevent disease such as hyaline membrane diseases, such as infant respiratory distress syndrome and bronchopulmonary dysplasia, in premature infants.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could stimulate the proliferation and differentiation of hepatocytes and, thus, could be used to alleviate or treat liver diseases and pathologies such as fulminant liver failure caused by cirrhosis, liver damage caused by viral hepatitis and toxic substances (i.e., acetaminophen, carbon tetrachloride and other hepatotoxins known in the art).

In addition, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to treat or prevent the onset of diabetes mellitus. In patients with newly diagnosed Types I and II diabetes, where some islet cell function remains, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used to maintain the islet function so as to alleviate, delay or prevent permanent manifestation of the disease. Also, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, could be used as an auxiliary in islet cell transplantation to improve or promote islet cell function.

#### **Neural Activity and Neurological Diseases**

The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used for the diagnosis and/or treatment of diseases, disorders, damage or injury of the brain and/or nervous system. Nervous system

disorders that can be treated with the compositions of the invention (e.g., fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention), include, but are not limited to, nervous system injuries, and diseases or disorders which result in either a disconnection of axons, a diminution or degeneration of neurons, or demyelination. Nervous system lesions which may be treated in a patient (including human and non-human mammalian patients) according to the methods of the invention, include but are not limited to, the following lesions of either the central (including spinal cord, brain) or peripheral nervous systems: (1) ischemic lesions, in which a lack of oxygen in a portion of the nervous system results in neuronal injury or death, including cerebral infarction or ischemia, or spinal cord infarction or ischemia; (2) traumatic lesions, including lesions caused by physical injury or associated with surgery, for example, lesions which sever a portion of the nervous system, or compression injuries; (3) malignant lesions, in which a portion of the nervous system is destroyed or injured by malignant tissue which is either a nervous system associated malignancy or a malignancy derived from non-nervous system tissue; (4) infectious lesions, in which a portion of the nervous system is destroyed or injured as a result of infection, for example, by an abscess or associated with infection by human immunodeficiency virus, herpes zoster, or herpes simplex virus or with Lyme disease, tuberculosis, or syphilis; (5) degenerative lesions, in which a portion of the nervous system is destroyed or injured as a result of a degenerative process including but not limited to, degeneration associated with Parkinson's disease, Alzheimer's disease, Huntington's chorea, or amyotrophic lateral sclerosis (ALS); (6) lesions associated with nutritional diseases or disorders, in which a portion of the nervous system is destroyed or injured by a nutritional disorder or disorder of metabolism including, but not limited to, vitamin B12 deficiency, folic acid deficiency, Wernicke disease, tobacco-alcohol amblyopia, Marchiafava-Bignami disease (primary degeneration of the corpus callosum), and alcoholic cerebellar degeneration; (7) neurological lesions associated with systemic diseases including, but not limited to, diabetes (diabetic neuropathy, Bell's palsy), systemic lupus erythematosus, carcinoma, or sarcoidosis; (8) lesions caused by toxic substances including alcohol, lead, or particular neurotoxins; and (9) demyelinated lesions in which a portion of the nervous system is destroyed or injured by a demyelinating disease including, but not limited to, multiple sclerosis, human immunodeficiency virus-associated myelopathy, transverse myelopathy or various

etiologies, progressive multifocal leukoencephalopathy, and central pontine myelinolysis.

In one embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to protect neural cells from the damaging effects of hypoxia. In a further preferred embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to protect neural cells from the damaging effects of cerebral hypoxia. According to this embodiment, the compositions of the invention are used to treat or prevent neural cell injury associated with cerebral hypoxia. In one non-exclusive aspect of this embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, are used to treat or prevent neural cell injury associated with cerebral ischemia. In another non-exclusive aspect of this embodiment, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent neural cell injury associated with cerebral infarction.

In another preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent neural cell injury associated with a stroke. In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent cerebral neural cell injury associated with a stroke.

In another preferred embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent neural cell injury associated with a heart attack. In a specific embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat or prevent cerebral neural cell injury associated with a heart attack.

The compositions of the invention which are useful for treating or preventing a nervous system disorder may be selected by testing for biological activity in promoting the survival or differentiation of neurons. For example, and not by way of limitation, compositions of the invention which elicit any of the following effects may be useful according to the invention: (1) increased survival time of neurons in culture either in the presence or absence of hypoxia or hypoxic conditions; (2) increased sprouting of neurons

in culture or *in vivo*; (3) increased production of a neuron-associated molecule in culture or *in vivo*, e.g., choline acetyltransferase or acetylcholinesterase with respect to motor neurons; or (4) decreased symptoms of neuron dysfunction *in vivo*. Such effects may be measured by any method known in the art. In preferred, non-limiting embodiments, increased survival of neurons may routinely be measured using a method set forth herein or otherwise known in the art, such as, for example, in Zhang *et al.*, *Proc Natl Acad Sci USA* 97:3637-42 (2000) or in Arakawa *et al.*, *J. Neurosci.*, 10:3507-15 (1990); increased sprouting of neurons may be detected by methods known in the art, such as, for example, the methods set forth in Pestronk *et al.*, *Exp. Neurol.*, 70:65-82 (1980), or Brown *et al.*, *Ann. Rev. Neurosci.*, 4:17-42 (1981); increased production of neuron-associated molecules may be measured by bioassay, enzymatic assay, antibody binding, Northern blot assay, etc., using techniques known in the art and depending on the molecule to be measured; and motor neuron dysfunction may be measured by assessing the physical manifestation of motor neuron disorder, e.g., weakness, motor neuron conduction velocity, or functional disability.

In specific embodiments, motor neuron disorders that may be treated according to the invention include, but are not limited to, disorders such as infarction, infection, exposure to toxin, trauma, surgical damage, degenerative disease or malignancy that may affect motor neurons as well as other components of the nervous system, as well as disorders that selectively affect neurons such as amyotrophic lateral sclerosis, and including, but not limited to, progressive spinal muscular atrophy, progressive bulbar palsy, primary lateral sclerosis, infantile and juvenile muscular atrophy, progressive bulbar paralysis of childhood (Fazio-Londe syndrome), poliomyelitis and the post polio syndrome, and Hereditary Motorsensory Neuropathy (Charcot-Marie-Tooth Disease).

Further, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may play a role in neuronal survival; synapse formation; conductance; neural differentiation, etc. Thus, compositions of the invention (including fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention) may be used to diagnose and/or treat or prevent diseases or disorders associated with these roles, including, but not limited to, learning and/or cognition disorders. The compositions of the invention may also be useful in the treatment or prevention of neurodegenerative disease states and/or behavioural disorders. Such

neurodegenerative disease states and/or behavioral disorders include, but are not limited to, Alzheimer's Disease, Parkinson's Disease, Huntington's Disease, Tourette Syndrome, schizophrenia, mania, dementia, paranoia, obsessive compulsive disorder, panic disorder, learning disabilities, ALS, psychoses, autism, and altered behaviors, including disorders in feeding, sleep patterns, balance, and perception. In addition, compositions of the invention may also play a role in the treatment, prevention and/or detection of developmental disorders associated with the developing embryo, or sexually-linked disorders.

Additionally, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be useful in protecting neural cells from diseases, damage, disorders, or injury, associated with cerebrovascular disorders including, but not limited to, carotid artery diseases (e.g., carotid artery thrombosis, carotid stenosis, or Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis (e.g., carotid artery thrombosis, sinus thrombosis, or Wallenberg's Syndrome), cerebral hemorrhage (e.g., epidural or subdural hematoma, or subarachnoid hemorrhage), cerebral infarction, cerebral ischemia (e.g., transient cerebral ischemia, Subclavian Steal Syndrome, or vertebrobasilar insufficiency), vascular dementia (e.g., multi-infarct), leukomalacia, periventricular, and vascular headache (e.g., cluster headache or migraines).

In accordance with yet a further aspect of the present invention, there is provided a process for utilizing fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, for therapeutic purposes, for example, to stimulate neurological cell proliferation and/or differentiation. Therefore, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used to treat and/or detect neurologic diseases. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used as a marker or detector of a particular nervous system disease or disorder.

Examples of neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, brain diseases, such as metabolic brain diseases which includes phenylketonuria such as maternal phenylketonuria, pyruvate carboxylase deficiency, pyruvate dehydrogenase complex deficiency, Wernicke's Encephalopathy, brain edema,

brain neoplasms such as cerebellar neoplasms which include infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms, supratentorial neoplasms, canavan disease, cerebellar diseases such as cerebellar ataxia which include spinocerebellar degeneration such as ataxia telangiectasia, cerebellar dyssynergia, Friederich's Ataxia, Machado-Joseph Disease, olivopontocerebellar atrophy, cerebellar neoplasms such as infratentorial neoplasms, diffuse cerebral sclerosis such as encephalitis periaxialis, globoid cell leukodystrophy, metachromatic leukodystrophy and subacute sclerosing panencephalitis.

Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include cerebrovascular disorders (such as carotid artery diseases which include carotid artery thrombosis, carotid stenosis and Moyamoya Disease), cerebral amyloid angiopathy, cerebral aneurysm, cerebral anoxia, cerebral arteriosclerosis, cerebral arteriovenous malformations, cerebral artery diseases, cerebral embolism and thrombosis such as carotid artery thrombosis, sinus thrombosis and Wallenberg's Syndrome, cerebral hemorrhage such as epidural hematoma, subdural hematoma and subarachnoid hemorrhage, cerebral infarction, cerebral ischemia such as transient cerebral ischemia, Subclavian Steal Syndrome and vertebrobasilar insufficiency, vascular dementia such as multi-infarct dementia, periventricular leukomalacia, vascular headache such as cluster headache and migraine.

Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include dementia such as AIDS Dementia Complex, presenile dementia such as Alzheimer's Disease and Creutzfeldt-Jakob Syndrome, senile dementia such as Alzheimer's Disease and progressive supranuclear palsy, vascular dementia such as multi-infarct dementia, encephalitis which include encephalitis periaxialis, viral encephalitis such as epidemic encephalitis, Japanese Encephalitis, St. Louis Encephalitis, tick-borne encephalitis and West Nile Fever, acute disseminated encephalomyelitis, meningoencephalitis such as uveomeningoencephalitic syndrome, Postencephalitic Parkinson Disease and subacute sclerosing panencephalitis, encephalomalacia such as periventricular leukomalacia, epilepsy such as generalized epilepsy which includes infantile spasms, absence epilepsy, myoclonic epilepsy which includes MERRF



Syndrome, tonic-clonic epilepsy, partial epilepsy such as complex partial epilepsy, frontal lobe epilepsy and temporal lobe epilepsy, post-traumatic epilepsy, status epilepticus such as Epilepsia Partialis Continua, and Hallervorden-Spatz Syndrome.

5 Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include hydrocephalus such as Dandy-Walker Syndrome and normal pressure hydrocephalus, hypothalamic diseases such as hypothalamic neoplasms, cerebral malaria, narcolepsy which includes cataplexy, bulbar poliomyelitis, cerebri pseudotumor, Rett Syndrome, Reye's Syndrome, thalamic diseases, cerebral toxoplasmosis, intracranial  
10 tuberculoma and Zellweger Syndrome, central nervous system infections such as AIDS Dementia Complex, Brain Abscess, subdural empyema, encephalomyelitis such as Equine Encephalomyelitis, Venezuelan Equine Encephalomyelitis, Necrotizing Hemorrhagic Encephalomyelitis, Visna, and cerebral malaria.

15 Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include meningitis such as arachnoiditis, aseptic meningitis such as viral meningitis which includes lymphocytic choriomeningitis, Bacterial meningitis which includes Haemophilus Meningitis, Listeria Meningitis, Meningococcal Meningitis such as Waterhouse-Friderichsen Syndrome, Pneumococcal Meningitis and meningeal  
20 tuberculosis, fungal meningitis such as Cryptococcal Meningitis, subdural effusion, meningoencephalitis such as uvemeningoencephalitic syndrome, myelitis such as transverse myelitis, neurosyphilis such as tabes dorsalis, poliomyelitis which includes bulbar poliomyelitis and postpoliomyelitis syndrome, prion diseases (such as Creutzfeldt-Jakob Syndrome, Bovine Spongiform Encephalopathy, Gerstmann-Straussler Syndrome, Kuru, Scrapie), and cerebral toxoplasmosis.  
25

Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include central nervous system neoplasms such as brain neoplasms that include cerebellar neoplasms such as infratentorial neoplasms, cerebral ventricle neoplasms such as choroid plexus neoplasms, hypothalamic neoplasms and supratentorial neoplasms,  
30 meningeal neoplasms, spinal cord neoplasms which include epidural neoplasms, demyelinating diseases such as Canavan Diseases, diffuse cerebral scleritis which includes

- adrenoleukodystrophy, encephalitis periaxialis, globoid cell leukodystrophy, diffuse cerebral sclerosis such as metachromatic leukodystrophy, allergic encephalomyelitis, necrotizing hemorrhagic encephalomyelitis, progressive multifocal leukoencephalopathy, multiple sclerosis, central pontine myelinolysis, transverse myelitis, neuromyelitis optica,
- 5 Scrapie, Swayback, Chronic Fatigue Syndrome, Visna, High Pressure Nervous Syndrome, Meningism, spinal cord diseases such as amyotonia congenita, amyotrophic lateral sclerosis, spinal muscular atrophy such as Werdnig-Hoffmann Disease, spinal cord compression, spinal cord neoplasms such as epidural neoplasms, syringomyelia, Tabes Dorsalis, Stiff-Man Syndrome, mental retardation such as Angelman Syndrome, Cri-du-
- 10 Chat Syndrome, De Lange's Syndrome, Down Syndrome, Gangliosidoses such as gangliosidoses G(M1), Sandhoff Disease, Tay-Sachs Disease, Hartnup Disease, homocystinuria, Laurence-Moon- Biedl Syndrome, Lesch-Nyhan Syndrome, Maple Syrup Urine Disease, mucopolidosis such as fucosidosis, neuronal ceroid-lipofuscinosis, oculocerebrorenal syndrome, phenylketonuria such as maternal phenylketonuria, Prader-
- 15 Willi Syndrome, Rett Syndrome, Rubinstein-Taybi Syndrome, Tuberous Sclerosis, WAGR Syndrome, nervous system abnormalities such as holoprosencephaly, neural tube defects such as anencephaly which includes hydrangencephaly, Arnold-Chairi Deformity, encephalocele, meningocele, meningomyelocele, spinal dysraphism such as spina bifida cystica and spina bifida occulta.
- 20 Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include hereditary motor and sensory neuropathies which include Charcot-Marie Disease, Hereditary optic atrophy, Refsum's Disease, hereditary spastic paraplegia, Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies such as
- 25 Congenital Analgesia and Familial Dysautonomia, Neurologic manifestations (such as agnosia that include Gerstmann's Syndrome, Amnesia such as retrograde amnesia, apraxia, neurogenic bladder, cataplexy, communicative disorders such as hearing disorders that includes deafness, partial hearing loss, loudness recruitment and tinnitus, language disorders such as aphasia which include agraphia, anomia, broca aphasia, and Wernicke
- 30 Aphasia, Dyslexia such as Acquired Dyslexia, language development disorders, speech disorders such as aphasia which includes anomia, broca aphasia and Wernicke Aphasia, articulation disorders, communicative disorders such as speech disorders which include

dysarthria, echolalia, mutism and stuttering, voice disorders such as aphonia and hoarseness, decerebrate state, delirium, fasciculation, hallucinations, meningism, movement disorders such as angelman syndrome, ataxia, athetosis, chorea, dystonia, hypokinesia, muscle hypotonia, myoclonus, tic, torticollis and tremor, muscle hypertonia

5 such as muscle rigidity such as stiff-man syndrome, muscle spasticity, paralysis such as facial paralysis which includes Herpes Zoster Oticus, Gastroparesis, Hemiplegia, ophthalmoplegia such as diplopia, Duane's Syndrome, Horner's Syndrome, Chronic progressive external ophthalmoplegia such as Kearns Syndrome, Bulbar Paralysis, Tropical Spastic Paraparesis, Paraplegia such as Brown-Sequard Syndrome, quadriplegia,

10 respiratory paralysis and vocal cord paralysis, paresis, phantom limb, taste disorders such as ageusia and dysgeusia, vision disorders such as amblyopia, blindness, color vision defects, diplopia, hemianopsia, scotoma and subnormal vision, sleep disorders such as hypersomnia which includes Kleine-Levin Syndrome, insomnia, and somnambulism, spasm such as trismus, unconsciousness such as coma, persistent vegetative state and

15 syncope and vertigo, neuromuscular diseases such as amyotonia congenita, amyotrophic lateral sclerosis, Lambert-Eaton Myasthenic Syndrome, motor neuron disease, muscular atrophy such as spinal muscular atrophy, Charcot-Marie Disease and Werdnig-Hoffmann Disease, Postpoliomyelitis Syndrome, Muscular Dystrophy, Myasthenia Gravis, Myotonia Atrophica, Myotonia Confenita, Nemaline Myopathy, Familial Periodic Paralysis,

20 Multiplex Paramyoclonus, Tropical Spastic Paraparesis and Stiff-Man Syndrome, peripheral nervous system diseases such as acrodynia, amyloid neuropathies, autonomic nervous system diseases such as Adie's Syndrome, Barre-Lieou Syndrome, Familial Dysautonomia, Horner's Syndrome, Reflex Sympathetic Dystrophy and Shy-Drager Syndrome, Cranial Nerve Diseases such as Acoustic Nerve Diseases such as Acoustic

25 Neuroma which includes Neurofibromatosis 2, Facial Nerve Diseases such as Facial Neuralgia, Melkersson-Rosenthal Syndrome, ocular motility disorders which includes amblyopia, nystagmus, oculomotor nerve paralysis, ophthalmoplegia such as Duane's Syndrome, Horner's Syndrome, Chronic Progressive External Ophthalmoplegia which includes Kearns Syndrome, Strabismus such as Esotropia and Exotropia, Oculomotor

30 Nerve Paralysis, Optic Nerve Diseases such as Optic Atrophy which includes Hereditary Optic Atrophy, Optic Disk Drusen, Optic Neuritis such as Neuromyelitis Optica, Papilledema, Trigeminal Neuralgia, Vocal Cord Paralysis, Demyelinating Diseases such as

Neuromyelitis Optica and Swayback, and Diabetic neuropathies such as diabetic foot.

Additional neurologic diseases which can be treated or detected with fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include nerve compression syndromes such as carpal tunnel syndrome, tarsal tunnel syndrome, thoracic outlet syndrome such as cervical rib syndrome, ulnar nerve compression syndrome, neuralgia such as causalgia, cervico-brachial neuralgia, facial neuralgia and trigeminal neuralgia, neuritis such as experimental allergic neuritis, optic neuritis, polyneuritis, polyradiculoneuritis and radiculitis such as polyradiculitis, hereditary motor and sensory neuropathies such as Charcot-Marie Disease, Hereditary Optic Atrophy, Refsum's Disease, Hereditary Spastic Paraplegia and Werdnig-Hoffmann Disease, Hereditary Sensory and Autonomic Neuropathies which include Congenital Analgesia and Familial Dysautonomia, POEMS Syndrome, Sciatica, Gustatory Sweating and Tetany).

#### **Endocrine Disorders**

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose disorders and/or diseases related to hormone imbalance, and/or disorders or diseases of the endocrine system.

Hormones secreted by the glands of the endocrine system control physical growth, sexual function, metabolism, and other functions. Disorders may be classified in two ways: disturbances in the production of hormones, and the inability of tissues to respond to hormones. The etiology of these hormone imbalance or endocrine system diseases, disorders or conditions may be genetic, somatic, such as cancer and some autoimmune diseases, acquired (e.g., by chemotherapy, injury or toxins), or infectious. Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used as a marker or detector of a particular disease or disorder related to the endocrine system and/or hormone imbalance.

Endocrine system and/or hormone imbalance and/or diseases encompass disorders of uterine motility including, but not limited to: complications with pregnancy and labor (e.g., pre-term labor, post-term pregnancy, spontaneous abortion, and slow or stopped

labor); and disorders and/or diseases of the menstrual cycle (e.g., dysmenorrhea and endometriosis).

Endocrine system and/or hormone imbalance disorders and/or diseases include disorders and/or diseases of the pancreas, such as, for example, diabetes mellitus, diabetes insipidus, congenital pancreatic agenesis, pheochromocytoma--islet cell tumor syndrome; disorders and/or diseases of the adrenal glands such as, for example, Addison's Disease, corticosteroid deficiency, virilizing disease, hirsutism, Cushing's Syndrome, hyperaldosteronism, pheochromocytoma; disorders and/or diseases of the pituitary gland, such as, for example, hyperpituitarism, hypopituitarism, pituitary dwarfism, pituitary adenoma, panhypopituitarism, acromegaly, gigantism; disorders and/or diseases of the thyroid, including but not limited to, hyperthyroidism, hypothyroidism, Plummer's disease, Graves' disease (toxic diffuse goiter), toxic nodular goiter, thyroiditis (Hashimoto's thyroiditis, subacute granulomatous thyroiditis, and silent lymphocytic thyroiditis), Pendred's syndrome, myxedema, cretinism, thyrotoxicosis, thyroid hormone coupling defect, thymic aplasia, Hurthle cell tumours of the thyroid, thyroid cancer, thyroid carcinoma, Medullary thyroid carcinoma; disorders and/or diseases of the parathyroid, such as, for example, hyperparathyroidism, hypoparathyroidism; disorders and/or diseases of the hypothalamus.

In addition, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases of the testes or ovaries, including cancer. Other disorders and/or diseases of the testes or ovaries further include, for example, ovarian cancer, polycystic ovary syndrome, Klinefelter's syndrome, vanishing testes syndrome (bilateral anorchia), congenital absence of Leydig's cells, cryptorchidism, Noonan's syndrome, myotonic dystrophy, capillary haemangioma of the testis (benign), neoplasias of the testis and neo-testis.

Moreover, endocrine system and/or hormone imbalance disorders and/or diseases may also include disorders and/or diseases such as, for example, polyglandular deficiency syndromes, pheochromocytoma, neuroblastoma, multiple Endocrine neoplasia, and disorders and/or cancers of endocrine tissues.

In another embodiment, albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to diagnose, prognose, prevent, and/or treat endocrine diseases and/or disorders associated

with the tissue(s) in which the Therapeutic protein corresponding to the Therapeutic protein portion of the albumin protein of the invention is expressed,

### **Reproductive System Disorders**

5       The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used for the diagnosis, treatment, or prevention of diseases and/or disorders of the reproductive system. Reproductive system disorders that can be treated by the compositions of the invention, include, but are not limited to, reproductive system injuries, infections, neoplastic disorders, congenital defects, and diseases or disorders which result in infertility, complications with pregnancy, labor, or parturition, and postpartum difficulties.

10       Reproductive system disorders and/or diseases include diseases and/or disorders of the testes, including testicular atrophy, testicular feminization, cryptorchism (unilateral and bilateral), anorchia, ectopic testis, epididymitis and orchitis (typically resulting from infections such as, for example, gonorrhea, mumps, tuberculosis, and syphilis), testicular torsion, vasitis nodosa, germ cell tumors (e.g., seminomas, embryonal cell carcinomas, teratocarcinomas, choriocarcinomas, yolk sac tumors, and teratomas), stromal tumors (e.g., Leydig cell tumors), hydrocele, hematocele, varicocele, spermatocele, inguinal hernia, and disorders of sperm production (e.g., immotile cilia syndrome, aspermia, asthenozoospermia, azoospermia, oligospermia, and teratozoospermia).

20       Reproductive system disorders also include disorders of the prostate gland, such as acute non-bacterial prostatitis, chronic non-bacterial prostatitis, acute bacterial prostatitis, chronic bacterial prostatitis, prostatodystonia, prostatosis, granulomatous prostatitis, malacoplakia, benign prostatic hypertrophy or hyperplasia, and prostate neoplastic disorders, including adenocarcinomas, transitional cell carcinomas, ductal carcinomas, and squamous cell carcinomas.

30       Additionally, the compositions of the invention may be useful in the diagnosis, treatment, and/or prevention of disorders or diseases of the penis and urethra, including inflammatory disorders, such as balanoposthitis, balanitis xerotica obliterans, phimosis, paraphimosis, syphilis, herpes simplex virus, gonorrhea, non-gonococcal urethritis, chlamydia, mycoplasma, trichomonas, HIV, AIDS, Reiter's syndrome, condyloma acuminatum, condyloma latum, and pearly penile papules; urethral abnormalities, such as

hypospadias, epispadias, and phimosis; premalignant lesions, including Erythroplasia of Queyrat, Bowen's disease, Bowenoid paplosis, giant condyloma of Buscke-Lowenstein, and verrucous carcinoma; penile cancers, including squamous cell carcinomas, carcinoma in situ, verrucous carcinoma, and disseminated penile carcinoma; urethral neoplastic disorders, including penile urethral carcinoma, bulbomembranous urethral carcinoma, and prostatic urethral carcinoma; and erectile disorders, such as priapism, Peyronie's disease, erectile dysfunction, and impotence.

Moreover, diseases and/or disorders of the vas deferens include vasculitis and CBAVD (congenital bilateral absence of the vas deferens); additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used in the diagnosis, treatment, and/or prevention of diseases and/or disorders of the seminal vesicles, including hydatid disease, congenital chloride diarrhea, and polycystic kidney disease.

Other disorders and/or diseases of the male reproductive system include, for example, Klinefelter's syndrome, Young's syndrome, premature ejaculation, diabetes mellitus, cystic fibrosis, Kartagener's syndrome, high fever, multiple sclerosis, and gynecomastia.

Further, the polynucleotides, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used in the diagnosis, treatment, and/or prevention of diseases and/or disorders of the vagina and vulva, including bacterial vaginosis, candida vaginitis, herpes simplex virus, chancroid, granuloma inguinale, lymphogranuloma venereum, scabies, human papillomavirus, vaginal trauma, vulvar trauma, adenosis, chlamydia vaginitis, gonorrhea, trichomonas vaginitis, condyloma acuminatum, syphilis, molluscum contagiosum, atrophic vaginitis, Paget's disease, lichen sclerosus, lichen planus, vulvodynia, toxic shock syndrome, vaginismus, vulvovaginitis, vulvar vestibulitis, and neoplastic disorders, such as squamous cell hyperplasia, clear cell carcinoma, basal cell carcinoma, melanomas, cancer of Bartholin's gland, and vulvar intraepithelial neoplasia.

Disorders and/or diseases of the uterus include dysmenorrhea, retroverted uterus, endometriosis, fibroids, adenomyosis, anovulatory bleeding, amenorrhea, Cushing's syndrome, hydatidiform moles, Asherman's syndrome, premature menopause, precocious puberty, uterine polyps, dysfunctional uterine bleeding (e.g., due to aberrant hormonal

signals), and neoplastic disorders, such as adenocarcinomas, leiomyosarcomas, and sarcomas. Additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be useful as a marker or detector of, as well as in the diagnosis, treatment, and/or prevention of congenital uterine abnormalities, such as bicornuate uterus, septate uterus, simple unicornuate uterus, unicornuate uterus with a noncavitary rudimentary horn, unicornuate uterus with a non-communicating cavitary rudimentary horn, unicornuate uterus with a communicating cavitary horn, arcuate uterus, uterine didelphys, and T-shaped uterus.

Ovarian diseases and/or disorders include anovulation, polycystic ovary syndrome (Stein-Leventhal syndrome), ovarian cysts, ovarian hypofunction, ovarian insensitivity to gonadotropins, ovarian overproduction of androgens, right ovarian vein syndrome, amenorrhea, hirsutism, and ovarian cancer (including, but not limited to, primary and secondary cancerous growth, Sertoli-Leydig tumors, endometrioid carcinoma of the ovary, ovarian papillary serous adenocarcinoma, ovarian mucinous adenocarcinoma, and Ovarian Krukenberg tumors).

Cervical diseases and/or disorders include cervicitis, chronic cervicitis, mucopurulent cervicitis, cervical dysplasia, cervical polyps, Nabothian cysts, cervical erosion, cervical incompetence, and cervical neoplasms (including, for example, cervical carcinoma, squamous metaplasia, squamous cell carcinoma, adenosquamous cell neoplasia, and columnar cell neoplasia).

Additionally, diseases and/or disorders of the reproductive system include disorders and/or diseases of pregnancy, including miscarriage and stillbirth, such as early abortion, late abortion, spontaneous abortion, induced abortion, therapeutic abortion, threatened abortion, missed abortion, incomplete abortion, complete abortion, habitual abortion, missed abortion, and septic abortion; ectopic pregnancy, anemia, Rh incompatibility, vaginal bleeding during pregnancy, gestational diabetes, intrauterine growth retardation, polyhydramnios, HELLP syndrome, abruptio placentae, placenta previa, hyperemesis, preeclampsia, eclampsia, herpes gestationis, and urticaria of pregnancy. Additionally, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may be used in the diagnosis, treatment, and/or prevention of diseases that can complicate pregnancy, including heart disease, heart failure, rheumatic heart disease, congenital heart disease,



mitral valve prolapse, high blood pressure, anemia, kidney disease, infectious disease (e.g., rubella, cytomegalovirus, toxoplasmosis, infectious hepatitis, chlamydia, HIV, AIDS, and genital herpes), diabetes mellitus, Graves' disease, thyroiditis, hypothyroidism, Hashimoto's thyroiditis, chronic active hepatitis, cirrhosis of the liver, primary biliary cirrhosis, asthma, systemic lupus eryematosus, rheumatoid arthritis, myasthenia gravis, idiopathic thrombocytopenic purpura, appendicitis, ovarian cysts, gallbladder disorders, and obstruction of the intestine.

Complications associated with labor and parturition include premature rupture of the membranes, pre-term labor, post-term pregnancy, postmaturity, labor that progresses too slowly, fetal distress (e.g., abnormal heart rate (fetal or maternal), breathing problems, and abnormal fetal position), shoulder dystocia, prolapsed umbilical cord, amniotic fluid embolism, and aberrant uterine bleeding.

Further, diseases and/or disorders of the postdelivery period, including endometritis, myometritis, parametritis, peritonitis, pelvic thrombophlebitis, pulmonary embolism, endotoxemia, pyelonephritis, saphenous thrombophlebitis, mastitis, cystitis, postpartum hemorrhage, and inverted uterus.

Other disorders and/or diseases of the female reproductive system that may be diagnosed, treated, and/or prevented by the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention include, for example, Turner's syndrome, pseudohermaphroditism, premenstrual syndrome, pelvic inflammatory disease, pelvic congestion (vascular engorgement), frigidity, anorgasmia, dyspareunia, ruptured fallopian tube, and Mittelschmerz.

## **Infectious Disease**

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used to treat or detect infectious agents. For example, by increasing the immune response, particularly increasing the proliferation and differentiation of B and/or T cells, infectious diseases may be treated. The immune response may be increased by either enhancing an existing immune response, or by initiating a new immune response. Alternatively, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also directly

inhibit the infectious agent, without necessarily eliciting an immune response.

Viruses are one example of an infectious agent that can cause disease or symptoms that can be treated or detected by albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention. Examples of viruses, include, but are not limited to Examples of viruses, include, but are not limited to the following DNA and RNA viruses and viral families: Arbovirus, Adenoviridae, Arenaviridae, Arterivirus, Birnaviridae, Bunyaviridae, Caliciviridae, Circoviridae, Coronaviridae, Dengue, EBV, HIV, Flaviviridae, Hepadnaviridae (Hepatitis), Herpesviridae (such as, Cytomegalovirus, Herpes Simplex, Herpes Zoster), Mononegavirus (e.g., Paramyxoviridae, Morbillivirus, Rhabdoviridae), Orthomyxoviridae (e.g., Influenza A, Influenza B, and parainfluenza), Papiloma virus, Papovaviridae, Parvoviridae, Picornaviridae, Poxviridae (such as Smallpox or Vaccinia), Reoviridae (e.g., Rotavirus), Retroviridae (HTLV-I, HTLV-II, Lentivirus), and Togaviridae (e.g., Rubivirus). Viruses falling within these families can cause a variety of diseases or symptoms, including, but not limited to: arthritis, bronchiolitis, respiratory syncytial virus, encephalitis, eye infections (e.g., conjunctivitis, keratitis), chronic fatigue syndrome, hepatitis (A, B, C, E, Chronic Active, Delta), Japanese B encephalitis, Junin, Chikungunya, Rift Valley fever, yellow fever, meningitis, opportunistic infections (e.g., AIDS), pneumonia, Burkitt's Lymphoma, chickenpox, hemorrhagic fever, Measles, Mumps, Parainfluenza, Rabies, the common cold, Polio, leukemia, Rubella, sexually transmitted diseases, skin diseases (e.g., Kaposi's, warts), and viremia. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used to treat or detect any of these symptoms or diseases. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat: meningitis, Dengue, EBV, and/or hepatitis (e.g., hepatitis B). In an additional specific embodiment fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat patients nonresponsive to one or more other commercially available hepatitis vaccines. In a further specific embodiment fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat AIDS.

Similarly, bacterial and fungal agents that can cause disease or symptoms and that can be treated or detected by albumin fusion proteins of the invention and/or

polynucleotides encoding albumin fusion proteins of the invention include, but not limited to, the following Gram-Negative and Gram-positive bacteria, bacterial families, and fungi: Actinomyces (e.g., Norcardia), Acinetobacter, *Cryptococcus neoformans*, Aspergillus, Bacillaceae (e.g., *Bacillus anthraxis*), Bacteroides (e.g., *Bacteroides fragilis*), Blastomycosis, Bordetella, Borrelia (e.g., *Borrelia burgdorferi*), Brucella, Candidia, Campylobacter, Chlamydia, Clostridium (e.g., *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens*, *Clostridium tetani*), Coccidioides, Corynebacterium (e.g., *Corynebacterium diphtheriae*), Cryptococcus, Dermatocycoses, *E. coli* (e.g., Enterotoxigenic *E. coli* and Enterohemorrhagic *E. coli*), Enterobacter (e.g. *Enterobacter aerogenes*), Enterobacteriaceae (Klebsiella, Salmonella (e.g., *Salmonella typhi*, *Salmonella enteritidis*, *Salmonella typhi*), Serratia, Yersinia, Shigella), Erysipelothrix, Haemophilus (e.g., *Haemophilus influenza* type B), Helicobacter, Legionella (e.g., *Legionella pneumophila*), Leptospira, Listeria (e.g., *Listeria monocytogenes*), Mycoplasma, Mycobacterium (e.g., *Mycobacterium leprae* and *Mycobacterium tuberculosis*), Vibrio (e.g., *Vibrio cholerae*), Neisseriaceae (e.g., *Neisseria gonorrhea*, *Neisseria meningitidis*), Pasteurellaceae, Proteus, Pseudomonas (e.g., *Pseudomonas aeruginosa*), Rickettsiaceae, Spirochetes (e.g., Treponema spp., Leptospira spp., Borrelia spp.), Shigella spp., Staphylococcus (e.g., *Staphylococcus aureus*), Meningioccus, Pneumococcus and Streptococcus (e.g., *Streptococcus pneumoniae* and Groups A, B, and C Streptococci), and Ureaplasmas. These bacterial, parasitic, and fungal families can cause diseases or symptoms, including, but not limited to: antibiotic-resistant infections, bacteremia, endocarditis, septicemia, eye infections (e.g., conjunctivitis), uveitis, tuberculosis, gingivitis, bacterial diarrhea, opportunistic infections (e.g., AIDS related infections), paronychia, prosthesis-related infections, dental caries, Reiter's Disease, respiratory tract infections, such as Whooping Cough or Empyema, sepsis, Lyme Disease, Cat-Scratch Disease, dysentery, paratyphoid fever, food poisoning, Legionella disease, chronic and acute inflammation, erythema, yeast infections, typhoid, pneumonia, gonorrhea, meningitis (e.g., meningitis types A and B), chlamydia, syphilis, diphtheria, leprosy, brucellosis, peptic ulcers, anthrax, spontaneous abortions, birth defects, pneumonia, lung infections, ear infections, deafness, blindness, lethargy, malaise, vomiting, chronic diarrhea, Crohn's disease, colitis, vaginosis, sterility, pelvic inflammatory diseases, candidiasis, paratuberculosis, tuberculosis, lupus, botulism,

gangrene, tetanus, impetigo, Rheumatic Fever, Scarlet Fever, sexually transmitted diseases, skin diseases (e.g., cellulitis, dermatocycoses), toxemia, urinary tract infections, wound infections, noscomial infections. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, can be used to treat or  
5 detect any of these symptoms or diseases. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat: tetanus, diptheria, botulism, and/or meningitis type B.

Moreover, parasitic agents causing disease or symptoms that can be treated, prevented, and/or diagnosed by fusion proteins of the invention and/or polynucleotides  
10 encoding albumin fusion proteins of the invention include, but not limited to, the following families or class: Amebiasis, Babesiosis, Coccidiosis, Cryptosporidiosis, Dientamoebiasis, Dourine, Ectoparasitic, Giardias, Helminthiasis, Leishmaniasis, Schistosoma, Theileriasis, Toxoplasmosis, Trypanosomiasis, and Trichomonas and Sporozoans (e.g., *Plasmodium virax*, *Plasmodium falciparium*, *Plasmodium malariae* and  
15 *Plasmodium ovale*). These parasites can cause a variety of diseases or symptoms, including, but not limited to: Scabies, Trombiculiasis, eye infections, intestinal disease (e.g., dysentery, giardiasis), liver disease, lung disease, opportunistic infections (e.g., AIDS related), malaria, pregnancy complications, and toxoplasmosis. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the  
20 invention, can be used to treat, prevent, and/or diagnose any of these symptoms or diseases. In specific embodiments, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention are used to treat, prevent, and/or diagnose malaria.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin  
25 fusion proteins of the invention could either be by administering an effective amount of an albumin fusion protein of the invention to the patient, or by removing cells from the patient, supplying the cells with a polynucleotide of the present invention, and returning the engineered cells to the patient (ex vivo therapy). Moreover, the albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the  
30 invention can be used as an antigen in a vaccine to raise an immune response against infectious disease.

## Regeneration

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention can be used to differentiate, proliferate, and attract cells, leading to the regeneration of tissues. (See, Science 276:59-87 (1997)). The regeneration of tissues could be used to repair, replace, or protect tissue damaged by congenital defects, trauma (wounds, burns, incisions, or ulcers), age, disease (e.g. osteoporosis, osteoarthritis, periodontal disease, liver failure), surgery, including cosmetic plastic surgery, fibrosis, reperfusion injury, or systemic cytokine damage.

Tissues that could be regenerated using the present invention include organs (e.g., pancreas, liver, intestine, kidney, skin, endothelium), muscle (smooth, skeletal or cardiac), vasculature (including vascular and lymphatics), nervous, hematopoietic, and skeletal (bone, cartilage, tendon, and ligament) tissue. Preferably, regeneration occurs without or decreased scarring. Regeneration also may include angiogenesis.

Moreover, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may increase regeneration of tissues difficult to heal. For example, increased tendon/ligament regeneration would quicken recovery time after damage. Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could also be used prophylactically in an effort to avoid damage. Specific diseases that could be treated include of tendinitis, carpal tunnel syndrome, and other tendon or ligament defects. A further example of tissue regeneration of non-healing wounds includes pressure ulcers, ulcers associated with vascular insufficiency, surgical, and traumatic wounds.

Similarly, nerve and brain tissue could also be regenerated by using fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, to proliferate and differentiate nerve cells. Diseases that could be treated using this method include central and peripheral nervous system diseases, neuropathies, or mechanical and traumatic disorders (e.g., spinal cord disorders, head trauma, cerebrovascular disease, and stroke). Specifically, diseases associated with peripheral nerve injuries, peripheral neuropathy (e.g., resulting from chemotherapy or other medical therapies), localized neuropathies, and central nervous system diseases (e.g., Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, and Shy-Drager syndrome), could all be treated using the albumin fusion proteins of the invention

and/or polynucleotides encoding albumin fusion proteins of the invention.

### **Gastrointestinal Disorders**

5           Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention, may be used to treat, prevent, diagnose, and/or prognose gastrointestinal disorders, including inflammatory diseases and/or conditions, infections, cancers (e.g., intestinal neoplasms (carcinoid tumor of the small intestine, non-Hodgkin's lymphoma of the small intestine, small bowel lymphoma)), and ulcers, such as peptic  
10   ulcers.

          Gastrointestinal disorders include dysphagia, odynophagia, inflammation of the esophagus, peptic esophagitis, gastric reflux, submucosal fibrosis and stricturing, Mallory-Weiss lesions, leiomyomas, lipomas, epidermal cancers, adeoncarcinomas, gastric retention disorders, gastroenteritis, gastric atrophy, gastric/stomach cancers, polyps of the  
15   stomach, autoimmune disorders such as pernicious anemia, pyloric stenosis, gastritis (bacterial, viral, eosinophilic, stress-induced, chronic erosive, atrophic, plasma cell, and Ménétrier's), and peritoneal diseases (e.g., chyloperitoneum, hemoperitoneum, mesenteric cyst, mesenteric lymphadenitis, mesenteric vascular occlusion, panniculitis, neoplasms, peritonitis, pneumoperitoneum, bubphrenic abscess,).

20           Gastrointestinal disorders also include disorders associated with the small intestine, such as malabsorption syndromes, distension, irritable bowel syndrome, sugar intolerance, celiac disease, duodenal ulcers, duodenitis, tropical sprue, Whipple's disease, intestinal lymphangiectasia, Crohn's disease, appendicitis, obstructions of the ileum, Meckel's diverticulum, multiple diverticula, failure of complete rotation of the small and large  
25   intestine, lymphoma, and bacterial and parasitic diseases (such as Traveler's diarrhea, typhoid and paratyphoid, cholera, infection by Roundworms (*Ascariasis lumbricoides*), Hookworms (*Ancylostoma duodenale*), Threadworms (*Enterobius vermicularis*), Tapeworms (*Taenia saginata*, *Echinococcus granulosus*, *Diphyllobothrium spp.*, and *T. solium*)).

30           Liver diseases and/or disorders include intrahepatic cholestasis (alagille syndrome, biliary liver cirrhosis), fatty liver (alcoholic fatty liver, reye syndrome), hepatic vein thrombosis, hepatolentricular degeneration, hepatomegaly, hepatopulmonary syndrome,

hepatorenal syndrome, portal hypertension (esophageal and gastric varices), liver abscess (amebic liver abscess), liver cirrhosis (alcoholic, biliary and experimental), alcoholic liver diseases (fatty liver, hepatitis, cirrhosis), parasitic (hepatic echinococcosis, fascioliasis, amebic liver abscess), jaundice (hemolytic, hepatocellular, and cholestatic), cholestasis, portal hypertension, liver enlargement, ascites, hepatitis (alcoholic hepatitis, animal hepatitis, chronic hepatitis (autoimmune, hepatitis B, hepatitis C, hepatitis D, drug induced), toxic hepatitis, viral human hepatitis (hepatitis A, hepatitis B, hepatitis C, hepatitis D, hepatitis E), Wilson's disease, granulomatous hepatitis, secondary biliary cirrhosis, hepatic encephalopathy, portal hypertension, varices, hepatic encephalopathy, primary biliary cirrhosis, primary sclerosing cholangitis, hepatocellular adenoma, hemangiomas, bile stones, liver failure (hepatic encephalopathy, acute liver failure), and liver neoplasms (angiomyolipoma, calcified liver metastases, cystic liver metastases, epithelial tumors, fibrolamellar hepatocarcinoma, focal nodular hyperplasia, hepatic adenoma, hepatobiliary cystadenoma, hepatoblastoma, hepatocellular carcinoma, hepatoma, liver cancer, liver hemangioendothelioma, mesenchymal hamartoma, mesenchymal tumors of liver, nodular regenerative hyperplasia, benign liver tumors (Hepatic cysts [Simple cysts, Polycystic liver disease, Hepatobiliary cystadenoma, Choledochal cyst], Mesenchymal tumors [Mesenchymal hamartoma, Infantile hemangioendothelioma, Hemangioma, Peliosis hepatis, Lipomas, Inflammatory pseudotumor, Miscellaneous], Epithelial tumors [Bile duct epithelium (Bile duct hamartoma, Bile duct adenoma), Hepatocyte (Adenoma, Focal nodular hyperplasia, Nodular regenerative hyperplasia)], malignant liver tumors [hepatocellular, hepatoblastoma, hepatocellular carcinoma, cholangiocellular, cholangiocarcinoma, cystadenocarcinoma, tumors of blood vessels, angiosarcoma, Kaposi's sarcoma, hemangioendothelioma, other tumors, embryonal sarcoma, fibrosarcoma, leiomyosarcoma, rhabdomyosarcoma, carcinosarcoma, teratoma, carcinoid, squamous carcinoma, primary lymphoma]), peliosis hepatis, erythrohepatic porphyria, hepatic porphyria (acute intermittent porphyria, porphyria cutanea tarda), Zellweger syndrome).

Pancreatic diseases and/or disorders include acute pancreatitis, chronic pancreatitis (acute necrotizing pancreatitis, alcoholic pancreatitis), neoplasms (adenocarcinoma of the pancreas, cystadenocarcinoma, insulinoma, gastrinoma, and glucagonoma, cystic neoplasms, islet-cell tumors, pancreoblastoma), and other pancreatic diseases (e.g., cystic

fibrosis, cyst (pancreatic pseudocyst, pancreatic fistula, insufficiency)).

Gallbladder diseases include gallstones (cholelithiasis and choledocholithiasis), postcholecystectomy syndrome, diverticulosis of the gallbladder, acute cholecystitis, chronic cholecystitis, bile duct tumors, and mucocele.

- 5 Diseases and/or disorders of the large intestine include antibiotic-associated colitis, diverticulitis, ulcerative colitis, acquired megacolon, abscesses, fungal and bacterial infections, anorectal disorders (e.g., fissures, hemorrhoids), colonic diseases (colitis, colonic neoplasms [colon cancer, adenomatous colon polyps (e.g., villous adenoma), colon carcinoma, colorectal cancer], colonic diverticulitis, colonic diverticulosis, megacolon
- 10 [Hirschsprung disease, toxic megacolon]; sigmoid diseases [proctocolitis, sigmoid neoplasms]), constipation, Crohn's disease, diarrhea (infantile diarrhea, dysentery), duodenal diseases (duodenal neoplasms, duodenal obstruction, duodenal ulcer, duodenitis), enteritis (enterocolitis), HIV enteropathy, ileal diseases (ileal neoplasms, ileitis), immunoproliferative small intestinal disease, inflammatory bowel disease
- 15 (ulcerative colitis, Crohn's disease), intestinal atresia, parasitic diseases (anisakiasis, balantidiasis, blastocystis infections, cryptosporidiosis, dientamoebiasis, amebic dysentery, giardiasis), intestinal fistula (rectal fistula), intestinal neoplasms (cecal neoplasms, colonic neoplasms, duodenal neoplasms, ileal neoplasms, intestinal polyps, jejunal neoplasms, rectal neoplasms), intestinal obstruction (afferent loop syndrome, duodenal obstruction,
- 20 impacted feces, intestinal pseudo-obstruction [cecal volvulus], intussusception), intestinal perforation, intestinal polyps (colonic polyps, gardner syndrome, peutz-jeghers syndrome), jejunal diseases (jejunal neoplasms), malabsorption syndromes (blind loop syndrome, celiac disease, lactose intolerance, short bowel syndrome, tropical sprue, whipple's disease), mesenteric vascular occlusion, pneumatosis cystoides intestinalis, protein-losing
- 25 enteropathies (intestinal lymphagiectasis), rectal diseases (anus diseases, fecal incontinence, hemorrhoids, proctitis, rectal fistula, rectal prolapse, rectocele), peptic ulcer (duodenal ulcer, peptic esophagitis, hemorrhage, perforation, stomach ulcer, Zollinger-Ellison syndrome), postgastrectomy syndromes (dumping syndrome), stomach diseases (e.g., achlorhydria, duodenogastric reflux (bile reflux), gastric antral vascular ectasia,
- 30 gastric fistula, gastric outlet obstruction, gastritis (atrophic or hypertrophic), gastroparesis, stomach dilatation, stomach diverticulum, stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, hyperplastic gastric polyp), stomach rupture, stomach



ulcer, stomach volvulus), tuberculosis, visceroptosis, vomiting (e.g., hematemesis, hyperemesis gravidarum, postoperative nausea and vomiting) and hemorrhagic colitis.

Further diseases and/or disorders of the gastrointestinal system include biliary tract diseases, such as, gastroschisis, fistula (e.g., biliary fistula, esophageal fistula, gastric fistula, intestinal fistula, pancreatic fistula), neoplasms (e.g., biliary tract neoplasms, esophageal neoplasms, such as adenocarcinoma of the esophagus, esophageal squamous cell carcinoma, gastrointestinal neoplasms, pancreatic neoplasms, such as adenocarcinoma of the pancreas, mucinous cystic neoplasm of the pancreas, pancreatic cystic neoplasms, pancreatoblastoma, and peritoneal neoplasms), esophageal disease (e.g., bullous diseases, candidiasis, glycogenic acanthosis, ulceration, barrett esophagus varices, atresia, cyst, diverticulum (e.g., Zenker's diverticulum), fistula (e.g., tracheoesophageal fistula), motility disorders (e.g., CREST syndrome, deglutition disorders, achalasia, spasm, gastroesophageal reflux), neoplasms, perforation (e.g., Boerhaave syndrome, Mallory-Weiss syndrome), stenosis, esophagitis, diaphragmatic hernia (e.g., hiatal hernia); gastrointestinal diseases, such as, gastroenteritis (e.g., cholera morbus, norwalk virus infection), hemorrhage (e.g., hematemesis, melena, peptic ulcer hemorrhage), stomach neoplasms (gastric cancer, gastric polyps, gastric adenocarcinoma, stomach cancer)), hernia (e.g., congenital diaphragmatic hernia, femoral hernia, inguinal hernia, obturator hernia, umbilical hernia, ventral hernia), and intestinal diseases (e.g., cecal diseases (appendicitis, cecal neoplasms)).

### Chemotaxis

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may have chemotaxis activity. A chemotaxic molecule attracts or mobilizes cells (e.g., monocytes, fibroblasts, neutrophils, T-cells, mast cells, eosinophils, epithelial and/or endothelial cells) to a particular site in the body, such as inflammation, infection, or site of hyperproliferation. The mobilized cells can then fight off and/or heal the particular trauma or abnormality.

Albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may increase chemotaxic activity of particular cells. These chemotactic molecules can then be used to treat inflammation, infection,

hyperproliferative disorders, or any immune system disorder by increasing the number of cells targeted to a particular location in the body. For example, chemotaxic molecules can be used to treat wounds and other trauma to tissues by attracting immune cells to the injured location. Chemotactic molecules of the present invention can also attract  
5 fibroblasts, which can be used to treat wounds.

It is also contemplated that fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may inhibit chemotactic activity. These molecules could also be used to treat disorders. Thus, fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention could be used as  
10 an inhibitor of chemotaxis.

### **Binding Activity**

Albumin fusion proteins of the invention may be used to screen for molecules that bind to the Therapeutic protein portion of the fusion protein or for molecules to which the  
15 Therapeutic protein portion of the fusion protein binds. The binding of the fusion protein and the molecule may activate (agonist), increase, inhibit (antagonist), or decrease activity of the fusion protein or the molecule bound. Examples of such molecules include antibodies, oligonucleotides, proteins (e.g., receptors), or small molecules.

Preferably, the molecule is closely related to the natural ligand of the Therapeutic  
20 protein portion of the fusion protein of the invention, e.g., a fragment of the ligand, or a natural substrate, a ligand, a structural or functional mimetic. (See, Coligan et al., Current Protocols in Immunology 1(2):Chapter 5 (1991)). Similarly, the molecule can be closely related to the natural receptor to which the Therapeutic protein portion of an albumin fusion protein of the invention binds, or at least, a fragment of the receptor capable of  
25 being bound by the Therapeutic protein portion of an albumin fusion protein of the invention (e.g., active site). In either case, the molecule can be rationally designed using known techniques.

Preferably, the screening for these molecules involves producing appropriate cells which express the albumin fusion proteins of the invention. Preferred cells include cells  
30 from mammals, yeast, *Drosophila*, or *E. coli*.

The assay may simply test binding of a candidate compound to an albumin fusion protein of the invention, wherein binding is detected by a label, or in an assay involving

competition with a labeled competitor. Further, the assay may test whether the candidate compound results in a signal generated by binding to the fusion protein.

Alternatively, the assay can be carried out using cell-free preparations, fusion protein/molecule affixed to a solid support, chemical libraries, or natural product mixtures.

- 5 The assay may also simply comprise the steps of mixing a candidate compound with a solution containing an albumin fusion protein, measuring fusion protein/molecule activity or binding, and comparing the fusion protein/molecule activity or binding to a standard.

- 10 Preferably, an ELISA assay can measure fusion protein level or activity in a sample (e.g., biological sample) using a monoclonal or polyclonal antibody. The antibody can measure fusion protein level or activity by either binding, directly or indirectly, to the albumin fusion protein or by competing with the albumin fusion protein for a substrate.

- 15 Additionally, the receptor to which a Therapeutic protein portion of an albumin fusion protein of the invention binds can be identified by numerous methods known to those of skill in the art, for example, ligand panning and FACS sorting (Coligan, et al., Current Protocols in Immun., 1(2), Chapter 5, (1991)). For example, in cases wherein the Therapeutic protein portion of the fusion protein corresponds to FGF, expression cloning may be employed wherein polyadenylated RNA is prepared from a cell responsive to the albumin fusion protein, for example, NIH3T3 cells which are known to contain multiple receptors for the FGF family proteins, and SC-3 cells, and a cDNA library created from  
20 this RNA is divided into pools and used to transfect COS cells or other cells that are not responsive to the albumin fusion protein. Transfected cells which are grown on glass slides are exposed to the albumin fusion protein of the present invention, after they have been labeled. The albumin fusion proteins can be labeled by a variety of means including iodination or inclusion of a recognition site for a site-specific protein kinase.

- 25 Following fixation and incubation, the slides are subjected to auto-radiographic analysis. Positive pools are identified and sub-pools are prepared and re-transfected using an iterative sub-pooling and re-screening process, eventually yielding a single clones that encodes the putative receptor.

- 30 As an alternative approach for receptor identification, a labeled albumin fusion protein can be photoaffinity linked with cell membrane or extract preparations that express the receptor molecule for the Therapeutic protein component of an albumin fusion protein of the invention, the linked material may be resolved by PAGE analysis and

exposed to X-ray film. The labeled complex containing the receptors of the fusion protein can be excised, resolved into peptide fragments, and subjected to protein microsequencing. The amino acid sequence obtained from microsequencing would be used to design a set of degenerate oligonucleotide probes to screen a cDNA library to identify the genes encoding the putative receptors.

Moreover, the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as "DNA shuffling") may be employed to modulate the activities of the fusion protein, and/or Therapeutic protein portion or albumin component of an albumin fusion protein of the present invention, thereby effectively generating agonists and antagonists of an albumin fusion protein of the present invention. See generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721, 5,834,252, and 5,837,458, and Patten, P. A., et al., *Curr. Opinion Biotechnol.* 8:724-33 (1997); Harayama, S. *Trends Biotechnol.* 16(2):76-82 (1998); Hansson, L. O., et al., *J. Mol. Biol.* 287:265-76 (1999); and Lorenzo, M. M. and Blasco, R. *Biotechniques* 24(2):308-13 (1998); each of these patents and publications are hereby incorporated by reference). In one embodiment, alteration of polynucleotides encoding albumin fusion proteins of the invention and thus, the albumin fusion proteins encoded thereby, may be achieved by DNA shuffling. DNA shuffling involves the assembly of two or more DNA segments into a desired molecule by homologous, or site-specific, recombination. In another embodiment, polynucleotides encoding albumin fusion proteins of the invention and thus, the albumin fusion proteins encoded thereby, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. In another embodiment, one or more components, motifs, sections, parts, domains, fragments, etc., of an albumin fusion protein of the present invention may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules. In preferred embodiments, the heterologous molecules are family members. In further preferred embodiments, the heterologous molecule is a growth factor such as, for example, platelet-derived growth factor (PDGF), insulin-like growth factor (IGF-I), transforming growth factor (TGF)-alpha, epidermal growth factor (EGF), fibroblast growth factor (FGF), TGF-beta, bone morphogenetic protein (BMP)-2, BMP-4, BMP-5, BMP-6, BMP-7, activins A and B, decapentaplegic(dpp), 60A, OP-2, dorsalin, growth differentiation factors (GDFs), nodal,

MIS, inhibin-alpha, TGF-beta1, TGF-beta2, TGF-beta3, TGF-beta5, and glial-derived neurotrophic factor (GDNF).

Other preferred fragments are biologically active fragments of the Therapeutic protein portion and/or albumin component of the albumin fusion proteins of the present invention. Biologically active fragments are those exhibiting activity similar, but not necessarily identical, to an activity of a Therapeutic protein portion and/or albumin component of the albumin fusion proteins of the present invention. The biological activity of the fragments may include an improved desired activity, or a decreased undesirable activity.

Additionally, this invention provides a method of screening compounds to identify those which modulate the action of an albumin fusion protein of the present invention. An example of such an assay comprises combining a mammalian fibroblast cell, an albumin fusion protein of the present invention, and the compound to be screened and  $^3\text{[H]}$  thymidine under cell culture conditions where the fibroblast cell would normally proliferate. A control assay may be performed in the absence of the compound to be screened and compared to the amount of fibroblast proliferation in the presence of the compound to determine if the compound stimulates proliferation by determining the uptake of  $^3\text{[H]}$  thymidine in each case. The amount of fibroblast cell proliferation is measured by liquid scintillation chromatography which measures the incorporation of  $^3\text{[H]}$  thymidine. Both agonist and antagonist compounds may be identified by this procedure.

In another method, a mammalian cell or membrane preparation expressing a receptor for the Therapeutic protein component of a fusion protein of the invention is incubated with a labeled fusion protein of the present invention in the presence of the compound. The ability of the compound to enhance or block this interaction could then be measured. Alternatively, the response of a known second messenger system following interaction of a compound to be screened and the receptor is measured and the ability of the compound to bind to the receptor and elicit a second messenger response is measured to determine if the compound is a potential fusion protein. Such second messenger systems include but are not limited to, cAMP guanylate cyclase, ion channels or phosphoinositide hydrolysis.

All of these above assays can be used as diagnostic or prognostic markers. The molecules discovered using these assays can be used to treat disease or to bring about a

particular result in a patient (e.g., blood vessel growth) by activating or inhibiting the fusion protein/molecule. Moreover, the assays can discover agents which may inhibit or enhance the production of the albumin fusion proteins of the invention from suitably manipulated cells or tissues.

5 Therefore, the invention includes a method of identifying compounds which bind to an albumin fusion protein of the invention comprising the steps of: (a) incubating a candidate binding compound with an albumin fusion protein of the present invention; and (b) determining if binding has occurred. Moreover, the invention includes a method of identifying agonists/antagonists comprising the steps of: (a) incubating a candidate  
10 compound with an albumin fusion protein of the present invention, (b) assaying a biological activity, and (b) determining if a biological activity of the fusion protein has been altered.

#### **Targeted Delivery**

15 In another embodiment, the invention provides a method of delivering compositions to targeted cells expressing a receptor for a component of an albumin fusion protein of the invention.

As discussed herein, fusion proteins of the invention may be associated with heterologous polypeptides, heterologous nucleic acids, toxins, or prodrugs via  
20 hydrophobic, hydrophilic, ionic and/or covalent interactions. In one embodiment, the invention provides a method for the specific delivery of compositions of the invention to cells by administering fusion proteins of the invention (including antibodies) that are associated with heterologous polypeptides or nucleic acids. In one example, the invention provides a method for delivering a Therapeutic protein into the targeted cell. In another  
25 example, the invention provides a method for delivering a single stranded nucleic acid (e.g., antisense or ribozymes) or double stranded nucleic acid (e.g., DNA that can integrate into the cell's genome or replicate episomally and that can be transcribed) into the targeted cell.

In another embodiment, the invention provides a method for the specific  
30 destruction of cells (e.g., the destruction of tumor cells) by administering an albumin fusion protein of the invention (e.g., polypeptides of the invention or antibodies of the invention) in association with toxins or cytotoxic prodrugs.

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By "toxin" is meant compounds that bind and activate endogenous cytotoxic effector systems, radioisotopes, holotoxins, modified toxins, catalytic subunits of toxins, or any molecules or enzymes not normally present in or on the surface of a cell that under defined conditions cause the cell's death. Toxins that may be used according to the methods of the invention include, but are not limited to, radioisotopes known in the art, compounds such as, for example, antibodies (or complement fixing containing portions thereof) that bind an inherent or induced endogenous cytotoxic effector system, thymidine kinase, endonuclease, RNase, alpha toxin, ricin, abrin, *Pseudomonas* exotoxin A, diphtheria toxin, saporin, momordin, gelonin, pokeweed antiviral protein, alpha-sarcin and cholera toxin. By "cytotoxic prodrug" is meant a non-toxic compound that is converted by an enzyme, normally present in the cell, into a cytotoxic compound. Cytotoxic prodrugs that may be used according to the methods of the invention include, but are not limited to, glutamyl derivatives of benzoic acid mustard alkylating agent, phosphate derivatives of etoposide or mitomycin C, cytosine arabinoside, daunorubisin, and phenoxyacetamide derivatives of doxorubicin.

#### Drug Screening

Further contemplated is the use of the albumin fusion proteins of the present invention, or the polynucleotides encoding these fusion proteins, to screen for molecules which modify the activities of the albumin fusion protein of the present invention or proteins corresponding to the Therapeutic protein portion of the albumin fusion protein. Such a method would include contacting the fusion protein with a selected compound(s) suspected of having antagonist or agonist activity, and assaying the activity of the fusion protein following binding.

This invention is particularly useful for screening therapeutic compounds by using the albumin fusion proteins of the present invention, or binding fragments thereof, in any of a variety of drug screening techniques. The albumin fusion protein employed in such a test may be affixed to a solid support, expressed on a cell surface, free in solution, or located intracellularly. One method of drug screening utilizes eukaryotic or prokaryotic host cells which are stably transformed with recombinant nucleic acids expressing the albumin fusion protein. Drugs are screened against such transformed cells or supernatants

obtained from culturing such cells, in competitive binding assays. One may measure, for example, the formulation of complexes between the agent being tested and an albumin fusion protein of the present invention.

Thus, the present invention provides methods of screening for drugs or any other  
5 agents which affect activities mediated by the albumin fusion proteins of the present invention. These methods comprise contacting such an agent with an albumin fusion protein of the present invention or a fragment thereof and assaying for the presence of a complex between the agent and the albumin fusion protein or a fragment thereof, by methods well known in the art. In such a competitive binding assay, the agents to screen  
10 are typically labeled. Following incubation, free agent is separated from that present in bound form, and the amount of free or uncomplexed label is a measure of the ability of a particular agent to bind to the albumin fusion protein of the present invention.

Another technique for drug screening provides high throughput screening for compounds having suitable binding affinity to an albumin fusion protein of the present  
15 invention, and is described in great detail in European Patent Application 84/03564, published on September 13, 1984, which is incorporated herein by reference herein. Briefly stated, large numbers of different small peptide test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The peptide test compounds are reacted with an albumin fusion protein of the present invention and washed. Bound  
20 peptides are then detected by methods well known in the art. Purified albumin fusion protein may be coated directly onto plates for use in the aforementioned drug screening techniques. In addition, non-neutralizing antibodies may be used to capture the peptide and immobilize it on the solid support.

This invention also contemplates the use of competitive drug screening assays in  
25 which neutralizing antibodies capable of binding an albumin fusion protein of the present invention specifically compete with a test compound for binding to the albumin fusion protein or fragments thereof. In this manner, the antibodies are used to detect the presence of any peptide which shares one or more antigenic epitopes with an albumin fusion protein of the invention.

30



## **Binding Peptides and Other Molecules**

The invention also encompasses screening methods for identifying polypeptides and nonpolypeptides that bind albumin fusion proteins of the invention, and the binding molecules identified thereby. These binding molecules are useful, for example, as  
5 agonists and antagonists of the albumin fusion proteins of the invention. Such agonists and antagonists can be used, in accordance with the invention, in the therapeutic embodiments described in detail, below.

This method comprises the steps of:

- contacting an albumin fusion protein of the invention with a plurality of molecules;
- 10 and
- identifying a molecule that binds the albumin fusion protein.

The step of contacting the albumin fusion protein of the invention with the plurality of molecules may be effected in a number of ways. For example, one may contemplate immobilizing the albumin fusion protein on a solid support and bringing a  
15 solution of the plurality of molecules in contact with the immobilized polypeptides. Such a procedure would be akin to an affinity chromatographic process, with the affinity matrix being comprised of the immobilized albumin fusion protein of the invention. The molecules having a selective affinity for the albumin fusion protein can then be purified by affinity selection. The nature of the solid support, process for attachment of the albumin  
20 fusion protein to the solid support, solvent, and conditions of the affinity isolation or selection are largely conventional and well known to those of ordinary skill in the art.

Alternatively, one may also separate a plurality of polypeptides into substantially separate fractions comprising a subset of or individual polypeptides. For instance, one can separate the plurality of polypeptides by gel electrophoresis, column chromatography, or  
25 like method known to those of ordinary skill for the separation of polypeptides. The individual polypeptides can also be produced by a transformed host cell in such a way as to be expressed on or about its outer surface (e.g., a recombinant phage). Individual isolates can then be "probed" by an albumin fusion protein of the invention, optionally in the presence of an inducer should one be required for expression, to determine if any  
30 selective affinity interaction takes place between the albumin fusion protein and the individual clone. Prior to contacting the albumin fusion protein with each fraction comprising individual polypeptides, the polypeptides could first be transferred to a solid

support for additional convenience. Such a solid support may simply be a piece of filter membrane, such as one made of nitrocellulose or nylon. In this manner, positive clones could be identified from a collection of transformed host cells of an expression library, which harbor a DNA construct encoding a polypeptide having a selective affinity for an albumin fusion protein of the invention. Furthermore, the amino acid sequence of the polypeptide having a selective affinity for an albumin fusion protein of the invention can be determined directly by conventional means or the coding sequence of the DNA encoding the polypeptide can frequently be determined more conveniently. The primary sequence can then be deduced from the corresponding DNA sequence. If the amino acid sequence is to be determined from the polypeptide itself, one may use microsequencing techniques. The sequencing technique may include mass spectroscopy.

In certain situations, it may be desirable to wash away any unbound polypeptides from a mixture of an albumin fusion protein of the invention and the plurality of polypeptides prior to attempting to determine or to detect the presence of a selective affinity interaction. Such a wash step may be particularly desirable when the albumin fusion protein of the invention or the plurality of polypeptides are bound to a solid support.

The plurality of molecules provided according to this method may be provided by way of diversity libraries, such as random or combinatorial peptide or nonpeptide libraries which can be screened for molecules that specifically bind an albumin fusion protein of the invention. Many libraries are known in the art that can be used, e.g., chemically synthesized libraries, recombinant (e.g., phage display libraries), and *in vitro* translation-based libraries. Examples of chemically synthesized libraries are described in Fodor et al., Science 251:767-773 (1991); Houghten et al., Nature 354:84-86 (1991); Lam et al., Nature 354:82-84 (1991); Medynski, Bio/Technology 12:709-710 (1994); Gallop et al., J. Medicinal Chemistry 37(9):1233-1251 (1994); Ohlmeyer et al., Proc. Natl. Acad. Sci. USA 90:10922-10926 (1993); Erb et al., Proc. Natl. Acad. Sci. USA 91:11422-11426 (1994); Houghten et al., Biotechniques 13:412 (1992); Jayawickreme et al., Proc. Natl. Acad. Sci. USA 91:1614-1618 (1994); Salmon et al., Proc. Natl. Acad. Sci. USA 90:11708-11712 (1993); PCT Publication No. WO 93/20242; and Brenner and Lerner, Proc. Natl. Acad. Sci. USA 89:5381-5383 (1992).

Examples of phage display libraries are described in Scott et al., Science 249:386-390 (1990); Devlin et al., Science, 249:404-406 (1990); Christian et al., 1992, J. Mol.

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Biol. 227:711-718 1992); Lenstra, J. Immunol. Meth. 152:149-157 (1992); Kay et al.,  
Gene 128:59-65 (1993); and PCT Publication No. WO 94/18318 dated Aug. 18, 1994.

In vitro translation-based libraries include but are not limited to those described in  
PCT Publication No. WO 91/05058 dated Apr. 18, 1991; and Mattheakis et al., Proc. Natl.  
5 Acad. Sci. USA 91:9022-9026 (1994).

By way of examples of nonpeptide libraries, a benzodiazepine library (see e.g.,  
Bunin et al., Proc. Natl. Acad. Sci. USA 91:4708-4712 (1994)) can be adapted for use.  
Peptoid libraries (Simon et al., Proc. Natl. Acad. Sci. USA 89:9367-9371 (1992)) can also  
be used. Another example of a library that can be used, in which the amide functionalities  
10 in peptides have been permethylated to generate a chemically transformed combinatorial  
library, is described by Ostresh et al. (Proc. Natl. Acad. Sci. USA 91:11138-11142  
(1994)).

The variety of non-peptide libraries that are useful in the present invention is great.  
For example, Ecker and Crooke (Bio/Technology 13:351-360 (1995)) list benzodiazepines,  
15 hydantoins, piperazinediones, biphenyls, sugar analogs, beta-mercaptoketones, arylacetic  
acids, acylpiperidines, benzopyrans, cubanes, xanthines, aminimides, and oxazolones as  
among the chemical species that form the basis of various libraries.

Non-peptide libraries can be classified broadly into two types: decorated  
monomers and oligomers. Decorated monomer libraries employ a relatively simple  
20 scaffold structure upon which a variety functional groups is added. Often the scaffold will  
be a molecule with a known useful pharmacological activity. For example, the scaffold  
might be the benzodiazepine structure.

Non-peptide oligomer libraries utilize a large number of monomers that are  
assembled together in ways that create new shapes that depend on the order of the  
25 monomers. Among the monomer units that have been used are carbamates, pyrrolinones,  
and morpholinos. Peptoids, peptide-like oligomers in which the side chain is attached to  
the alpha amino group rather than the alpha carbon, form the basis of another version of  
non-peptide oligomer libraries. The first non-peptide oligomer libraries utilized a single  
type of monomer and thus contained a repeating backbone. Recent libraries have utilized  
30 more than one monomer, giving the libraries added flexibility.

Screening the libraries can be accomplished by any of a variety of commonly  
known methods. See, e.g., the following references, which disclose screening of peptide

libraries: Parmley et al., Adv. Exp. Med. Biol. 251:215-218 (1989); Scott et al., Science 249:386-390 (1990); Fowlkes et al., BioTechniques 13:422-427 (1992); Oldenburg et al., Proc. Natl. Acad. Sci. USA 89:5393-5397 (1992); Yu et al., Cell 76:933-945 (1994); Staudt et al., Science 241:577-580 (1988); Bock et al., Nature 355:564-566 (1992); Tuerk et al., Proc. Natl. Acad. Sci. USA 89:6988-6992 (1992); Ellington et al., Nature 355:850-852 (1992); U.S. Pat. No. 5,096,815, U.S. Pat. No. 5,223,409, and U.S. Pat. No. 5,198,346, all to Ladner et al.; Rebar et al., Science 263:671-673 (1993); and PCT Publication No. WO 94/18318.

In a specific embodiment, screening to identify a molecule that binds an albumin fusion protein of the invention can be carried out by contacting the library members with an albumin fusion protein of the invention immobilized on a solid phase and harvesting those library members that bind to the albumin fusion protein. Examples of such screening methods, termed "panning" techniques are described by way of example in Parmley et al., Gene 73:305-318 (1988); Fowlkes et al., BioTechniques 13:422-427 (1992); PCT Publication No. WO 94/18318; and in references cited herein.

In another embodiment, the two-hybrid system for selecting interacting proteins in yeast (Fields et al., Nature 340:245-246 (1989); Chien et al., Proc. Natl. Acad. Sci. USA 88:9578-9582 (1991) can be used to identify molecules that specifically bind to polypeptides of the invention.

Where the binding molecule is a polypeptide, the polypeptide can be conveniently selected from any peptide library, including random peptide libraries, combinatorial peptide libraries, or biased peptide libraries. The term "biased" is used herein to mean that the method of generating the library is manipulated so as to restrict one or more parameters that govern the diversity of the resulting collection of molecules, in this case peptides.

Thus, a truly random peptide library would generate a collection of peptides in which the probability of finding a particular amino acid at a given position of the peptide is the same for all 20 amino acids. A bias can be introduced into the library, however, by specifying, for example, that a lysine occur every fifth amino acid or that positions 4, 8, and 9 of a decapeptide library be fixed to include only arginine. Clearly, many types of biases can be contemplated, and the present invention is not restricted to any particular bias. Furthermore, the present invention contemplates specific types of peptide libraries,

such as phage displayed peptide libraries and those that utilize a DNA construct comprising a lambda phage vector with a DNA insert.

As mentioned above, in the case of a binding molecule that is a polypeptide, the polypeptide may have about 6 to less than about 60 amino acid residues, preferably about 6 to about 10 amino acid residues, and most preferably, about 6 to about 22 amino acids. In another embodiment, a binding polypeptide has in the range of 15-100 amino acids, or 20-50 amino acids.

The selected binding polypeptide can be obtained by chemical synthesis or recombinant expression.

### **Other Activities**

An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention, may be employed in treatment for stimulating re-vascularization of ischemic tissues due to various disease conditions such as thrombosis, arteriosclerosis, and other cardiovascular conditions. The albumin fusion proteins of the invention and/or polynucleotides encoding albumin fusion proteins of the invention may also be employed to stimulate angiogenesis and limb regeneration, as discussed above.

An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed for treating wounds due to injuries, burns, post-operative tissue repair, and ulcers since they are mitogenic to various cells of different origins, such as fibroblast cells and skeletal muscle cells, and therefore, facilitate the repair or replacement of damaged or diseased tissue.

An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed stimulate neuronal growth and to treat and prevent neuronal damage which occurs in certain neuronal disorders or neuro-degenerative conditions such as Alzheimer's disease, Parkinson's disease, and AIDS-related complex. An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may have the ability to stimulate chondrocyte growth, therefore, they may be employed to enhance bone and periodontal regeneration and aid in tissue transplants or bone grafts.

An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be also be employed to prevent skin aging due

to sunburn by stimulating keratinocyte growth.

An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed for preventing hair loss, since FGF family members activate hair-forming cells and promotes melanocyte growth.

- 5 Along the same lines, an albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be employed to stimulate growth and differentiation of hematopoietic cells and bone marrow cells when used in combination with other cytokines.

- 10 An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed to maintain organs before transplantation or for supporting cell culture of primary tissues. An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be employed for inducing tissue of mesodermal origin to differentiate in early embryos.

- 15 An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also increase or decrease the differentiation or proliferation of embryonic stem cells, besides, as discussed above, hematopoietic lineage.

- 20 An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be used to modulate mammalian characteristics, such as body height, weight, hair color, eye color, skin, percentage of adipose tissue, pigmentation, size, and shape (e.g., cosmetic surgery). Similarly, an albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be used to modulate mammalian metabolism affecting catabolism, anabolism, processing, utilization, and storage of energy.

- 25 An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may be used to change a mammal's mental state or physical state by influencing biorhythms, cardiac rhythms, depression (including depressive disorders), tendency for violence, tolerance for pain, reproductive capabilities (preferably by Activin or Inhibin-like activity), hormonal or endocrine levels, appetite, 30 libido, memory, stress, or other cognitive qualities.

An albumin fusion protein of the invention and/or polynucleotide encoding an albumin fusion protein of the invention may also be used as a food additive or

preservative, such as to increase or decrease storage capabilities, fat content, lipid, protein, carbohydrate, vitamins, minerals, cofactors or other nutritional components.

The above-recited applications have uses in a wide variety of hosts. Such hosts include, but are not limited to, human, murine, rabbit, goat, guinea pig, camel, horse, mouse, rat, hamster, pig, micro-pig, chicken, goat, cow, sheep, dog, cat, non-human primate; and human. In specific embodiments, the host is a mouse, rabbit, goat, guinea pig, chicken, rat, hamster, pig, sheep, dog or cat. In preferred embodiments, the host is a mammal. In most preferred embodiments, the host is a human.

Having generally described the invention, the same will be more readily understood by reference to the following examples, which are provided by way of illustration and are not intended as limiting.

Without further description, it is believed that one of ordinary skill in the art can, using the preceding description and the following illustrative examples, make and utilize the alterations detected in the present invention and practice the claimed methods. The following working examples therefore, specifically point out preferred embodiments of the present invention, and are not to be construed as limiting in any way the remainder of the disclosure.

## EXAMPLES

### *Example 1: Preparation of HA-hGH Fusion Proteins*

An HA-hGH fusion protein was prepared as follows:

#### Cloning of hGH cDNA

The hGH cDNA was obtained from a human pituitary gland cDNA library (catalogue number HL1097v, Clontech Laboratories, Inc) by PCR amplification. Two oligonucleotides suitable for PCR amplification of the hGH cDNA, HGH1 and HGH2, were synthesized using an Applied Biosystems 380B Oligonucleotide Synthesizer.

HGH1: 5' - CCCAAGAATTCCCTTATCCAGGC - 3' (SEQ ID NO: 1)

HGH2: 5' - GGGAAGCTTAGAAGCCACAGGATCCCTCCACAG - 3' (SEQ ID

NO: 2)

HGH 1 and HGH2 differed from the equivalent portion of the hGH cDNA sequence (Martial *et. al.*, 1979) by two and three nucleotides, respectively, such that after PCR amplification an *EcoRI* site would be introduced to the 5' end of the cDNA and a *BamHI* site would be introduced into the 3' end of the cDNA. In addition, HGH2 contained a *HindIII* site immediately downstream of the hGH sequence.

PCR amplification using a Perkin-Elmer-Cetus Thermal Cycler 9600 and a Perkin-Elmer-Cetus PCR kit, was performed using single-stranded DNA template isolated from the phage particles of the cDNA library as follows: 10 µL phage particles were lysed by the addition of 10 µL phage lysis buffer (280 µg/mL proteinase K in TE buffer) and incubation at 55°C for 15 min followed by 85°C for 15 min. After a 1 min. incubation on ice, phage debris was pelleted by centrifugation at 14,000 rpm for 3 min. The PCR mixture contained 6 µL of this DNA template, 0.1 µM of each primer and 200 µM of each deoxyribonucleotide. PCR was carried out for 30 cycles, denaturing at 94°C for 30 s, annealing at 65°C for 30 s and extending at 72°C for 30 s, increasing the extension time by 1 s per cycle.

Analysis of the reaction by gel electrophoresis showed a single product of the expected size (589 base pairs).

The PCR product was purified using Wizard PCR Preps DNA Purification System (Promega Corp) and then digested with *EcoRI* and *HindIII*. After further purification of the *EcoRI-HindIII* fragment by gel electrophoresis, the product was cloned into pUC19 (GIBCO BRL) digested with *EcoRI* and *HindIII*, to give pHGH1. DNA sequencing of the *EcoRI HindIII* region showed that the PCR product was identical in sequence to the hGH sequence (Martial *et al.*, 1979), except at the 5' and 3' ends, where the *EcoRI* and *BamHI* sites had been introduced, respectively.

#### *Expression of the hGH cDNA.*

The polylinker sequence of the phagemid pBluescribe (+) (Stratagene) was replaced by inserting an oligonucleotide linker, formed by annealing two 75-mer oligonucleotides, between the *EcoRI* and *HindIII* sites to form pBST(+). The new polylinker included a unique *NotI* site.

The *NotI* HA expression cassette of pAYE309 (EP 431 880) comprising the PRBI



promoter, DNA encoding the HA/MF -I hybrid leader sequence, DNA encoding HA and the ADH1 terminator, was transferred to pBST(+) to form pHA1. The HA coding sequence was removed from this plasmid by digestion with HindIII followed by religation to form pHA2.

5 Cloning of the hGH cDNA, as described in Example 1, provided the hGH coding region lacking the pro-hGH sequence and the first 8 base pairs (bp) of the mature hGH sequence. In order to construct an expression plasmid for secretion of hGH from yeast, a yeast promoter, signal peptide and the first 8 bp of the hGH sequence were attached to the 5' end of the cloned hGH sequence as follows: The *HindIII*-*SfaNI* fragment from pHA 1  
10 was attached to the 5' end of the *EcoRI*/*HindIII* fragment from pHGH1 via two synthetic oligonucleotides, HGH3 and HGH4 (which can anneal to one another in such a way as to generate a double stranded fragment of DNA with sticky ends that can anneal with *SfaNI* and *EcoRI* sticky ends):

HGH3: 5' - GATAAAGATTCCCAAC - 3' (SEQ ID NO: 3)

15 HGH4: 5' - AATTGTTGGGAATCTTT- 3' (SEQ ID NO: 4)

The *HindIII* fragment so formed was cloned into *HindIII*-digested pHA2 to make pHGH2, such that the hGH cDNA was positioned downstream of the PRBI promoter and HA/MF -1 fusion leader sequence (WO 90/01063). The *NotI* expression cassette contained in pHGH2, which included the *ADH1* terminator downstream of the hGH  
20 cDNA, was cloned into *NotI*-digested pSAC35 (Sleep *et al.*, BioTechnology 8:42 (1990)) to make pHGH12. This plasmid comprised the entire 2  $\mu$ m plasmid to provide replication functions and the LEU2 gene for selection of transformants.

pHGH12 was introduced into *S. cerevisiae* D88 by transformation and individual transformants were grown for 3 days at 30°C in 10 mL YEPD (1% w/v yeast extract, 2 %  
25 w/v, peptone, 2 % w/v, dextrose).

After centrifugation of the cells, the supernatants were examined by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and were found to contain protein which was of the expected size and which was recognized by anti-hGH antiserum (Sigma, Poole, UK) on Western blots.

30

#### *Cloning and expression of an HA-hGH fusion protein.*

In order to fuse the HA cDNA to the 5' end of the hGH cDNA, the pHA1

*HindIII*-*Bsu361* fragment (containing most of the *HA* cDNA) was joined to the pHGH1 *EcoRI*-*HindIII* fragment (containing most of the hGH cDNA) via two oligonucleotides, HGH7 and HGH8

HGH7: 5' - TTAGGCTTATTCCCAAC 3' (SEQ ID NO: 5)

5 HGH8: 5' - AATTGTTGGGAATAAGCC 3' (SEQ ID NO: 6)

The *HindIII* fragment so formed was cloned into pHA2 digested with *HindIII* to make pHGH10, and the *NotI* expression cassette of this plasmid was cloned into *NotI*-digested pSAC35 to make pHGH16.

pHGH16 was used to transform *S. cerevisiae* D88 and supernatants of cultures  
10 were analyzed as described above. A predominant band was observed that had a molecular weight of approximately 88 kD, corresponding to the combined masses of HA and hGH. Western blotting using anti-*HA* and anti-hGH antisera (Sigma) confirmed the presence of the two constituent parts of the albumin fusion protein.

The albumin fusion protein was purified from culture supernatant by cation  
15 exchange chromatography, followed by anion exchange and gel permeation chromatography. Analysis of the N-terminus of the protein by amino acid sequencing confirmed the presence of the expected albumin sequence.

An *in vitro* growth hormone activity assay (Ealey *et al.*, Growth Regulation 5:36 (1995)) indicated that the albumin fusion protein possessed full hGH activity. In a  
20 hypophysectomised rat weight gain model, performed essentially as described in the European Pharmacopoeia (1987, monograph 556), the fusion molecule was more potent than hGH when the same number of units of activity (based on the above *in vitro* assay) were administered daily. Further experiments in which the albumin fusion protein was administered once every four days showed a similar overall growth response to a daily  
25 administration of hGH. Pharmacokinetic experiments in which <sup>125</sup>I- labeled protein was administered to rats indicated an approximately ten-fold increase in circulatory half-life for the albumin fusion protein compared to hGH.

A similar plasmid was constructed in which DNA encoding the *S. cerevisiae* invertase (SUC2) leader sequence replaced the sequence for the hybrid leader, such that  
30 the encoded leader and the junction (↓) with the HA sequence were as follows:

... MLLQAFLLLAGFAAKISA ↓ DAHKS ..... (SEQ ID NO: 7) Invertase leader  
HA sequence ...

On introduction into *S. cerevisiae* DBI, this plasmid directed the expression and secretion of the albumin fusion protein at a level similar to that obtained with pHGH16. Analysis of the N-terminus of the albumin fusion protein indicated precise and efficient  
5 cleavage of the leader sequence from the mature protein.

*Cloning and expression of an hGH-HA fusion protein.*

In order to fuse the hGH cDNA to the 5' end of the HA cDNA, the HA cDNA was first altered by site-directed mutagenesis to introduce an *Eco*NI site near the 5' end of the  
10 coding region. This was done by the method of Kunkel *et al.* (Methods in Enzymol. 154:367 (1987)) using single-stranded DNA template prepared from pHAI and a synthetic oligonucleotide, LEU4:

LEU4: 5' - GAGATGCACACCTGAGTGAGG - 3' (SEQ ID NO: 8)

Site-directed mutagenesis using this oligonucleotide changed the coding sequence  
15 of the HA cDNA from Lys4 to Leu4 (K4L). However, this change was repaired when the hGH cDNA was subsequently joined at the 5' end by linking the pHGH2 *Not*I-*Bam*HI fragment to the *Eco*NI-*Not*I fragment of the mutated pHAI, via the two oligonucleotides HGH5 and HGH6:

HGH5: 5' - GATCCTGTGGCTTCGATGCACACAAGA - 3' (SEQ ID NO: 9)

20 HGH6: 5' - CTCTTGTGTGCATCGAAGCCACAG - 3' (SEQ ID NO: 10)

The *Not*I fragment so formed was cloned into *Not*I-digested pSAC35 to make pHGH14. pHGH14 was used to transform *S. cerevisiae* D88 and supernatants of culture were analyzed as above. A predominant band was observed that had a molecular weight of approximately 88 kD, corresponding to the combined masses of hGH and HA. Western  
25 blotting using anti-HA and anti-hGH antisera confirmed the presence of the two constituent parts of the albumin fusion protein.

The albumin fusion protein was purified from culture supernatant by cation exchange chromatography, followed by anion exchange and gel permeation chromatography. Analysis of the N-terminus of the protein by amino acid sequencing  
30 confirmed the presence of the expected hGH sequence.

*In vitro* studies showed that the albumin fusion protein retained hGH activity, but was significantly less potent than an albumin fusion protein comprising full length HA

(1-585) as the N-terminal portion and hGH as the C-terminal portion, as described above.

*Construction of plasmids for the expression of hGH fusions to domains of HA.*

Fusion polypeptides were made in which the hGH molecule was fused to the first two domains of HA (residues 1 to 387). Fusion to the N terminus of hGH was achieved by joining the pHA1 *HindIII*-*SapI* fragment, which contained most of the coding sequence for domains 1 and 2 of HA, to the pHGH1 *EcoRI*-*HindIII* fragment, via the oligonucleotides HGH 11 and HGH 12:

HGH11: 5' - TGTGGAAGAGCCTCAGAATTTATTCCCAAC - 3' (SEQ ID NO:

10 11)

HGH12: 5' - AATTGTTGGGAATAAATTCTGAGGCTCTTCC - 3' (SEQ ID NO: 12)

The *HindIII* fragment so formed was cloned into *HindIII*-digested pHA2 to make pHGH37 and the *NotI* expression cassette of this plasmid was cloned into *NotI*-digested pSAC35.

The resulting plasmid, pHGH38, contained an expression cassette that was found to direct secretion of the fusion polypeptide into the supernatant when transformed into *S. cerevisiae* DB 1. Western blotting using anti-*HA* and anti-hGH antisera confirmed the presence of the two constituent parts of the albumin fusion protein.

The albumin fusion protein was purified from culture supernatant by cation exchange chromatography followed by gel permeation chromatography.

*In vivo* studies with purified protein indicated that the circulatory half-life was longer than that of hGH, and similar to that of an albumin fusion protein comprising full-length HA (1-585) as the N-terminal portion and hGH as the C-terminal portion, as described above. *In vitro* studies showed that the albumin fusion protein retained hGH activity.

Using a similar strategy as detailed above, an albumin fusion protein comprising the first domain of HA (residues 1-194) as the N-terminal portion and hGH as the C-terminal portion, was cloned and expressed in *S. cerevisiae* DBL. Western blotting of culture supernatant using anti-*HA* and anti-hGH antisera confirmed the presence of the two constituent parts of the albumin fusion protein.

*Fusion of HA to hGH using a flexible linker sequence*

Flexible linkers, comprising repeating units of [Gly-Gly-Gly-Gly-Ser]<sub>n</sub>, where n was either 2 or 3, were introduced between the HA and hGH albumin fusion protein by cloning of the oligonucleotides HGH16, HGH17, HGH18 and HGH19:

5 HGH16:5'-TTAGGCTTAGGTGGCGGTGGATCCGGCGGTGGTGGATCTTTC  
CCA AC-3' (SEQ ID NO: 13)

HGH17:5'-AATTGTTGGGAAAGATCCACCACCGCCGGATCCACCGCCACC  
TAAGCC-3' (SEQ ID NO: 14)

HGH18:5'-TTAGGCTTAGGCGGTGGTGGATCTGGTGGCGGCGGATCTGGT  
10 GGCGGTGGATCCTTCCCAAC-3' (SEQ ID NO: 15)

HGH19:  
5'-AATTGTTGGGAAGGATCCACCGCCACCAGATCCGCCGCCACCA  
GATCCACCACCGCCTAAGCC-3' (SEQ ID NO: 16)

Annealing of HGH16 with HGH17 resulted in n=2, while HGH18 annealed to  
15 HGH19 resulted in n=3. After annealing, the double-stranded oligonucleotides were  
cloned with the *EcoRI*-*Bsu361* fragment isolated from pHGH1 into *Bsu361*-digested  
pHGH10 to make pHGH56 (where n=2) and pHGH57 (where n=3). The *NotI* expression  
cassettes from these plasmids were cloned into *NotI*-digested pSAC35 to make pHGH58  
and pHGH59, respectively.

20 Cloning of the oligonucleotides to make pHGH56 and pHGH57 introduced a  
*Bam*HI site in the linker sequences. It was therefore possible to construct linker sequences  
in which n=1 and n = 4, by joining either the *Hind*III-*Bam*HI fragment from pHGH56 to  
the *Bam*HI-*Hind*III fragment from pHGH57 (making n = 1), or the *Hind*III-*Bam*HI  
fragment from pHGH57 to the *Bam*HI-*Hind*III fragment from pHGH56 (making n=2).  
25 Cloning of these fragments into the *Hind*III site of pHA2, resulted in pHGH60 (n= 1) and  
pHGH61 (n=4). The *NotI* expression cassettes from pHGH60 and pHGH61 were cloned  
into *NotI*-digested pSAC35 to make pHGH62 and pHGH63, respectively.

Transformation of *S. cerevisiae* with pHGH58, pHGH59, pHGH62 and pHGH63  
resulted in transformants that secreted the fusion polypeptides into the supernatant.  
30 Western blotting using anti-HA and anti-hGH antisera confirmed the presence of the two  
constituent parts of the albumin fusion proteins.

The albumin fusion proteins were purified from culture supernatant by cation

exchange chromatography, followed by anion exchange and gel permeation chromatography. Analysis of the N-termini of the proteins by amino acid sequencing confirmed the presence of the expected albumin sequence. Analysis of the purified proteins by electrospray mass spectrometry confirmed an increase in mass of 315 D (n=1), 630 D (n=2), 945 D (n=3) and 1260 D (n=4) compared to the HA-hGH fusion protein described above. The purified protein was found to be active *in vitro*.

#### Increased Shelf-Life of HA-hGH fusion proteins: Methods

HA-hGH and hGH were separately diluted in cell culture media containing 5% horse serum to final concentrations of 100-200 µg/ml and incubated at 4, 37 or 50°C. At time zero and at weekly intervals thereafter, aliquots of the samples were tested for their biological activity in the Nb2 cell proliferation assay, and the data normalized to the biological activity of the control (hGH solution at time zero). In other assays hGH and HA-hGH were incubated in phosphate buffer saline in at 4, 37 and 50 degree C.

Nb2 cell proliferation assay: The growth of these cells is dependent on hGH or other lactogenic hormones. In a typical experiment  $10^4$  cells /well are plated in 96-well plate in the presence of different concentration of hGH or HA-hGH in media such as DMEM containing 5-10% horse serum for 24-48 hrs in the incubator. After the incubation period, 1:10 volume of MTT (5mg/ml in H<sub>2</sub>O) is added to each well and the plate is incubated for a further 6-16 hrs. The growing cells convert MTT to insoluble formazan. The formazan is solubilized by acidic isopropanol, and the color produced is measured at 570 nm on microtiter plate reader. The extent of formazan formation reflects the level of cellular proliferation.

#### Increased shelf-life of HA-hGH fusion proteins: Results

The fusion of Therapeutic proteins to albumin confers stability in aqueous or other solution. Figure 1 depicts the extended shelf-life of an HA fusion protein in terms of the biological activity of HA-hGH remaining after storage in cell culture media for up to 5 weeks at 37°C. A solution of 200 µg/ml HA-hGH was prepared in tissue culture media containing 5% horse serum, and the solution incubated at 37°C starting at time zero. At the indicated times, a sample was removed and tested for its biological activity in the Nb2 cell assay, at 2 ng/ml final concentration. As shown in Figure 1, the biological activity of

HA-hGH remains essentially intact (within experimental variation) after 5 weeks of incubation at 37°C. The recombinant hGH used as control for this experiment lost its biological activity in the first week of the experiment.

Figure 2 shows the stability of HA-hGH after storage in cell culture media for up to 3 weeks at 4, 37, or 50°C. At time zero, a solution of HA-hGH was prepared in tissue culture media containing 5% horse serum, and incubated at 4, 37, and 50°C. At the indicated periods a sample was removed and assayed for its biological activity in the Nb2 cell proliferation assay, at 60 ng/ml final concentration. HA-hGH retains over 90% of its initial activity at all temperatures tested for at least 3 weeks after incubation while hGH loses its biological activity within the first week. This level of activity is further retained for at least 7 weeks at 37° C and 5 weeks at 50° C. These results indicate that HA-hGH is highly stable in aqueous solution even under temperature stress.

Figures 3A and 3B show the stable biological activity of HA-hGH compared to hGH in the Nb2 cell proliferation assay. Nb2 cells were grown in the presence of increasing concentrations of recombinant hGH or HA-hGH, added at time zero. The cells were incubated for 24 or 48 hours before measuring the extent of proliferation by the MTT method. The increased stability of HA-hGH in the assay results in essentially the same proliferative activity at 24 hours (Figure 3A) as at 48 hours (Figure 3B) while hGH shows a significant reduction in its proliferative activity after 48 hours of incubation (Figures 3A and 3B). Compared to hGH, the HA-hGH has lower biological potency after 1 day; the albumin fusion protein is about 5 fold less potent than hGH. However, after 2 days the HA-hGH shows essentially the same potency as hGH due to the short life of hGH in the assay. This increase in the stability of the hGH as an albumin fusion protein has a major unexpected impact on the biological activity of the protein. Although the potency of the albumin fusion proteins is slightly lower than the unfused counterparts in rapid bioassays, their biological stability results in much higher biological activity in the longer term *in vitro* assay or *in vivo* assays.

#### ***Example 2: Preparation of HA-fusion proteins.***

Figure 4 shows a map of a plasmid (pPPC0005) that can be used as the base vector for cloning the cDNAs of therapeutic partners to form HA-fusions. For example, digestion of this vector with the restriction enzymes *Bsu36I*/Partial *HindIII* will allow for

the insertion of a cDNA modified at the 5' end to encode the last 5 amino acids of HA including the *Bsu36I* site and at the 3' end to include a double stop codon and *HindIII* site.

As another example, digestion of this vector with the restriction enzymes *Bsu36I*, *SphI* allows for the insertion of a cDNA modified at the 5' end to encode the last 5 amino acids of HA including the *Bsu36I* site and at the 3' end to include a double stop codon, *HindIII* site and the *ADHI* terminator sequence up to and including the *SphI* site.

This plasmid may easily be modified by one of skill in the art, for example, to modify, add or delete restriction sites so that one may more easily clone a Therapeutic protein, or fragment or variant of into the vector for the purpose of making an albumin fusion protein of the invention.

For example, for the purpose of making an albumin fusion protein where the Therapeutic moiety is placed N-terminal to the (mature) albumin protein, restriction sites were added at the 5' end of the DNA encoding HA in pPPC0005 shown in Figure 4).

Because it was desired to add unique *XhoI* and *ClaI* sites at the 5' end of the DNA encoding the HA protein in pPPC0005, it was first necessary to remove those same sites from the plasmid (located 3' of the *ADHI* terminator sequence). This was accomplished by cutting pPPC0005 with *XhoI* and *ClaI*, filling in the sticky ends with T4 DNA polymerase, and religating the blunt ends to create pPPC0006

Engineering the *Xho* and *Cla I* restriction sites into the Fusion leader sequence just 5' of the DNA encoding the HA protein in pPPC0006 was accomplished using two rounds of PCR. The first pair of oligonucleotides are those of SEQ ID NO:19 and SEQ ID NO:20. SEQ ID 19 contains four point mutations relative to the DNA sequence encoding the Fusion leader sequence and the beginning of the HA protein. These mutations are necessary to create the *XhoI* site in the fusion leader sequence and the *Cla I* site just at the beginning of the DNA encoding the HA protein. These four mutations are underlined in the sequence shown below. In pPPC0006 the nucleotides at these four positions from 5' to 3' are T, G, T, and G.

5'-GCCTCGAGAAAAGAGATGCACACAAGAGTGAGGTTGCTCATCGATTTAAAG  
ATTGGG-3' (SEQ ID NO:19)

5'-AATCGATGAGCAACCTCACTCTTGTGTGCATCTCTTTCTCGAGGCTCCTGG  
AATAAGC-3' (SEQ ID NO:20). A second round of PCR is then performed with an



upstream flanking primer, 5'-TACAAACTTAAGAGTCCAATTAGC-3' (SEQ ID NO:21) and a downstream flanking primer 5'-CACTTCTCTAGAGTGGTTTCATATGTCTT-3' (SEQ ID NO:22). The resulting PCR product is then purified and then digested with AflII and XbaI and ligated into the same sites in pPPC0006 creating pScCHSA. The resulting plasmid will have an XhoI sites engineered into the fusion leader sequence. The presence of the XhoI site creates a single amino acid change in the end of fusion leader sequence from LDKR to LEKR. The D to E change will not be present in the final albumin fusion protein expression plasmid if one ligates into the XhoI and Cla I sites a fragment comprising the Therapeutic moiety which has a 5' SalI sticky end (which is compatible with the XhoI end) and a 3' ClaI end. Ligation of the XhoI to the SalI restores the original amino acid sequence of the Fusion leader sequence. The therapeutic protein moiety may be inserted after the Kex2 site (Kex2 cleaves after the dibasic amino acid sequence KR at the end of the Fusion leader sequence) and before the ClaI site.

In addition, for the purpose of making an albumin fusion protein where the Therapeutic moiety is placed C-terminal to the (mature) albumin protein, four, eight-base-pair restriction sites were added at the 3' end of the DNA encoding HA in pScCHSA. As an example, it was felt to be desirable to incorporate AscI, FseI, and PmeI restriction sites in between the Bsu36I and HindIII sites at the end of the DNA encoding the HA protein in pScCHSA. This was accomplished through the use of two complementary synthetic oligonucleotides (SEQ ID NO:19 and SEQ ID NO:20) which contain the desired restriction sites.

5'-AAGCTGCCTTAGGCTTATAATAAGGCGCGCCGGCCGGCCGTTTAAACTAAGCTTAATTCT-3' (SEQ ID NO:23) and

5'-AGAATTAAGCTTAGTTTAAACGGCCGGCCGGCGCGCCTTATTATAAGCCTAAGGCAGCTT-3' (SEQ ID NO:24). These oligonucleotides may be annealed and digested with Bsu36I and HindIII and ligated into the same sites located at the end of the DNA encoding the HA protein in pScCHSA creating pScNHSA, using techniques known in the art.

*Making vectors comprising albumin fusion proteins where the albumin moiety is N-terminal to the Therapeutic moiety.*

The DNA encoding the Therapeutic moiety may be PCR amplified using primers

that will add DNA encoding the last five amino acids of the HA (and containing the Bsu36I site) onto the 5' end of the DNA encoding a Therapeutic protein and a STOP codon and appropriate cloning sites onto the 3' end of the coding sequence. For instance, the forward primer used to amplify the DNA encoding a therapeutic protein might have the sequence, 5'-aagctGCCTTAGGCTTA(N)<sub>15</sub>-3' (SEQ ID NO:25) where the underlined sequence is a Bsu36I site, the upper case nucleotides encode the last four amino acids of the mature HA protein (ALGL), and (N)<sub>15</sub> is identical to the first 15 nucleotides encoding the Therapeutic protein of interest. Similarly, the reverse primer used to amplify the DNA encoding a therapeutic protein might have the sequence, 5'-GCGCGCGTTTAAACGGCCGGCCGGCGCGCCTTATTA(N)<sub>15</sub>-3' (SEQ ID NO:26) where the italicized nucleotides is a PmeI site, the double underlined nucleotides are a FseI site, the singly underlined text is a PmeI site, the boxed nucleotides are the reverse complement of two tandem stop codons, and (N)<sub>15</sub> is identical to the reverse complement of the last 15 nucleotides encoding the Therapeutic protein of interest. Once the PCR product is amplified it may be cut with Bsu36I and one of (AscI, FseI, or PmeI) and ligated into pScNHSA.

*Making vectors comprising albumin fusion proteins where the albumin moiety is N-terminal to the Therapeutic moiety.*

The DNA encoding the Therapeutic moiety may be PCR amplified using primers that will add DNA encoding the last three amino acids of the Fusion leader sequence (and containing a SalI site) onto the 5' end of the DNA encoding a Therapeutic protein and the first few amino acids of the HA (and containing a ClaI site. For instance, the forward primer used to amplify the DNA encoding a therapeutic protein might have the sequence, 5'-aggagcgtcGACAAAAGA(N)<sub>15</sub>-3' (SEQ ID NO:27) where the underlined sequence is a Sal I site, the upper case nucleotides encode the last three amino acids of the Fusion leader sequence (DKR), and (N)<sub>15</sub> is identical to the first 15 nucleotides encoding the Therapeutic protein of interest. Similarly, the reverse primer used to amplify the DNA encoding a therapeutic protein might have the sequence, 5'-CTTTAAATCGATGAGCAACCTCACTCTTGTGTGCATC(N)<sub>15</sub>-3' (SEQ ID NO:28) where the italicized nucleotides are a ClaI site, the underlined nucleotides are the reverse complement of the DNA encoding the first 9 amino acids of HA (DAHKSEVAH), and

(N)<sub>15</sub> is identical to the reverse complement of the last 15 nucleotides encoding the Therapeutic protein of interest. Once the PCR product is amplified it may be cut with Sall and ClaI and ligated into pScCHSA digested with XhoI and Cla I.

5 *Expression of an Albumin Fusion Protein in yeast.*

The Not I fragment containing the DNA encoding either an N-terminal or C-terminal albumin fusion protein generated from pScCHSA or pScNHSA may then be cloned in to the NotI site of pSAC35.

10 *Expression of an Albumin Fusion Protein from Mammalian cell lines*

The HSA gene has also been cloned into a the pC4 vector which is more suitable for mammalian culture systems creating plasmid pC4:HSA. More specifically, pC4HSA was generated by PCR amplifying the mature HSA gene with a 5' primer (SEQ ID NO:30) that anneals to the 5' end of DNA encoding the mature form of the HSA protein (e.g, DNA in plasmid pScCHSA), incorporates BamHI (Shown in italics below) and HindIII (shown singly underlined below) cloning sites, attaches a kozak sequence (shown double underlined below) and DNA encoding the natural HSA signal peptide (MKWVSFISLLFLFSSAYSRS�DKR, SEQ ID NO:29) (shown in bold below), and a 3' primer (SEQ ID NO:31) that anneals to the 3' end of DNA encoding the mature form of the HSA protein and incorporates an Asp718 restriction site (shown in bold below). The DNA encoding the natural human serum albumin leader sequence in SEQ ID NO:30 also contains a modification that introduces a XhoI site that is boxed below.

5'-TCAGGGATCCAAGCTTCCGCCACCATGAAGTGGGTAACCTTTATTTCCCTTC  
TTTTTCTCTTTAGCTCGGCTTA<sup>CTCGAG</sup>GGGTGTGTTTCGTCGAGATGCACACA  
AGAGTGAG-3' (SEQ ID NO:30)

5"-GCAGCGGTACCGAATTCGGCGCGCCTTATAAGCCTAAGGCAGC-3' (SEQ ID NO:31)

30

This PCR product (1.85kb) is then purified and digested with BamHI and Asp718 and cloned into the same sites in pC4 (ATCC Accession No. 209646) to produce pC4:HSA

*Making vectors comprising albumin fusion proteins where the albumin moiety is C-terminal to the Therapeutic moiety using the pC4:HSA vector*

Using pC4:HSA, albumin fusion proteins in which the Therapeutic protein moiety is N terminal to the albumin sequence, one can clone DNA encoding a Therapeutic protein that has its own signal sequence between the Bam HI (or HindIII) and ClaI sites. When cloning into either the BamHI or Hind III site remember to include Kozak sequence (CCGCCACCATG) prior to translational start codon of DNA encoding the Therapeutic Protein to be subcloned. If the Therapeutic does not have a signal sequence, the DNA encoding that Therapeutic protein may be cloned in between the XhoI and ClaI sites. When using the XhoI site, the following 5' (SEQ ID NO:32) and 3' (SEQ IDNO:33) PCR primers may be used:

5'-CCGCCGCTCGAGGGGTGTGTTTCGTCGA(N)<sub>18</sub>-3' (SEQ ID NO: 32)

5'-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATC(N)<sub>18</sub>-3' (SEQ ID NO:33)

In SEQ ID NO:32, the underlined sequence is an XhoI site; and the XhoI site and the DNA following the XhoI site encode for the last seven amino acids of the leader sequence of natural human serum albumin. In SEQ ID NO:33, the underlined sequence is a ClaI site; and the ClaI site and the DNA following it encode are the reverse complement of the DNA encoding the first 10 amino acids of themature HSA protein (SEQ ID NO:18). In SEQ ID NO:32 "(N)<sub>18</sub>" is DNA identical to the first 18 nucleotides encoding the Therapeutic protein of interest.). In SEQ ID NO:33 "(N)<sub>18</sub>" is the reverse complement of DNA encoding the last 18 nucleotides encoding the Therapeutic protein of interest. Using these two primers, one may PCR amplify the Therapeutic protein of interest, purify the PCR product, digest it with XhoI and ClaI restriction enzymes and then and clone it into the with XhoI and ClaI sites in the pC4:HSA vector:

*Making vectors comprising albumin fusion proteins where the albumin moiety is N-terminal to the Therapeutic moiety using the pC4:HSA vector*

Using pC4:HSA, albumin fusion proteins in which the Therapeutic protein moiety is N terminal to the albumin sequence, one can clone DNA encoding a Therapeutic protein between the Bsu36I and AscI restriction sites. When cloning into the Bsu36I and AscI, the

same primer design used to clone in the yeast vector system (SEQ ID NO:25 and 26) may be employed.

The pC4 vector is especially suitable for expression of albumin fusion proteins from CHO cells. For expression, in other mammalian cell types, e.g., NSO cells, it may be useful to subclone the HindIII - EcoRI fragment containing the DNA encoding an albumin fusion protein (from a pC4 vector in which the DNA encoding the Therapeutic protein has already been cloned in frame with the DNA encoding (the mature form of) human serum albumin) into another expression vector (such as any of the mammalian expression vectors described herein).

***Example 3: Preparation of HA-cytokine or HA-growth factor fusion proteins (such as EPO, GMCSF, GCSF)***

The cDNA for the cytokine or growth factor of interest, such as EPO, can be isolated by a variety of means including from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for all of these proteins are known and available, for instance, in U.S. Patents 4,703,008, 4,810,643 and 5,908,763. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. EPO (or other cytokine) cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA, or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines, a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

***Example 4: Preparation of HA-IFN fusion proteins (such as IFN $\alpha$ )***

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The cDNA for the interferon of interest such as IFN $\alpha$  can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for interferons, such as IFN $\alpha$  are known and available, for instance, in U.S. Patents 5,326,859 and 4,588,585, in EP 32 134, as well as in public databases such as GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used to clone the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus of the HA sequence, with or without the use of a spacer sequence. The IFN $\alpha$  (or other interferon) cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA, or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast (see Figure 8). The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

20 Maximum protein recovery from vials

The albumin fusion proteins of the invention have a high degree of stability even when they are packaged at low concentrations. In addition, in spite of the low protein concentration, good fusion-protein recovery is observed even when the aqueous solution includes no other protein added to minimize binding to the vial walls. Figure 5 compares the recovery of vial-stored HA-IFN solutions with a stock solution. 6 or 30  $\mu$ g/ml HA-IFN solutions were placed in vials and stored at 4°C. After 48 or 72 hrs a volume originally equivalent to 10 ng of sample was removed and measured in an IFN sandwich ELISA. The estimated values were compared to that of a high concentration stock solution. As shown, there is essentially no loss of the sample in these vials, indicating that addition of exogenous material such as albumin is not necessary to prevent sample loss to the wall of the vials

*In vivo stability and bioavailability of HA- $\alpha$ -IFN fusions*

To determine the in vivo stability and bioavailability of a HA- $\alpha$ -IFN fusion molecule, the purified fusion molecule (from yeast) was administered to monkeys at the dosages and time points described in Figures 6 and 7. Pharmaceutical compositions formulated from HA- $\alpha$ -IFN fusions may account for the extended serum half-life and bioavailability exemplified in Figures 6 and 7. Accordingly, pharmaceutical compositions may be formulated to contain lower dosages of alpha-interferon activity compared to the native alpha-interferon molecule.

Pharmaceutical compositions containing HA- $\alpha$ -IFN fusions may be used to treat or prevent disease in patients with any disease or disease state that can be modulated by the administration of  $\alpha$ -IFN. Such diseases include, but are not limited to, hairy cell leukemia, Kaposi's sarcoma, genital and anal warts, chronic hepatitis B, chronic non-A, non-B hepatitis, in particular hepatitis C, hepatitis D, chronic myelogenous leukemia, renal cell carcinoma, bladder carcinoma, ovarian and cervical carcinoma, skin cancers, recurrent respirator papillomatosis, non-Hodgkin's and cutaneous T-cell lymphomas, melanoma, multiple myeloma, AIDS, multiple sclerosis, glioblastoma, etc. (see Interferon Alpha, In: AHFS Drug Information, 1997).

Accordingly, the invention includes pharmaceutical compositions containing a HA- $\alpha$ -IFN fusion protein, polypeptide or peptide formulated with the proper dosage for human administration. The invention also includes methods of treating patients in need of such treatment comprising at least the step of administering a pharmaceutical composition containing at least one HA- $\alpha$ -IFN fusion protein, polypeptide or peptide.

*Bifunctional HA- $\alpha$ -IFN fusions*

The HA- $\alpha$ -IFN expression vector of Figure 8 is modified to include an insertion for the expression of bifunctional HA- $\alpha$ -IFN fusion proteins. For instance, the cDNA for a second protein of interest may be inserted in frame downstream of the "rHA-IFN" sequence after the double stop codon has been removed or shifted downstream of the coding sequence.

In one version of a bifunctional HA- $\alpha$ -IFN fusion protein, an antibody or fragment against B-lymphocyte stimulator protein (GenBank Acc 4455139) or polypeptide may be fused to one end of the HA component of the fusion molecule. This bifunctional protein is

useful for modulating any immune response generated by the  $\alpha$ -IFN component of the fusion.

**Example 5: Preparation of HA-hormone fusion protein (such as insulin, LH, FSH)**

The cDNA for the hormone of interest such as insulin can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for all of these proteins are known and available, for instance, in public databases such as GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The hormone cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA, or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

**Example 6: Preparation of HA-soluble receptor or HA-binding protein fusion protein such as HA-TNF receptor**

The cDNA for the soluble receptor or binding protein of interest such as TNF receptor can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for all of these proteins are known and available, for instance, in GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The receptor cDNA is cloned



into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

***Example 7: Preparation of HA-growth factors such as HA-IGF-1 fusion protein***

The cDNA for the growth factor of interest such as IGF-1 can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods (see GenBank Acc. No.NP\_000609). The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The growth factor cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

***Example 8: Preparation of HA-single chain antibody fusion proteins***

Single chain antibodies are produced by several methods including but not limited to: selection from phage libraries, cloning of the variable region of a specific antibody by cloning the cDNA of the antibody and using the flanking constant regions as the primer to

clone the variable region, or by synthesizing an oligonucleotide corresponding to the variable region of any specific antibody. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The cell cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA, or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast.

In fusion molecules of the invention, the  $V_H$  and  $V_L$  can be linked by one of the following means or a combination thereof: a peptide linker between the C-terminus of the  $V_H$  and the N-terminus of the  $V_L$ ; a Kex2p protease cleavage site between the  $V_H$  and  $V_L$  such that the two are cleaved apart upon secretion and then self associate; and cystine residues positioned such that the  $V_H$  and  $V_L$  can form a disulphide bond between them to link them together (see Figure 14). An alternative option would be to place the  $V_H$  at the N-terminus of HA or an HA domain fragment and the  $V_L$  at the C-terminus of the HA or HA domain fragment.

The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines. The antibody produced in this manner can be purified from media and tested for its binding to its antigen using standard immunochemical methods.

#### ***Example 9: Preparation of HA-cell adhesion molecule fusion proteins***

The cDNA for the cell adhesion molecule of interest can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The nucleotide sequences for the known cell adhesion molecules are known and available, for instance, in GenBank. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or

without the use of a spacer sequence. The cell adhesion molecule cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

***Example 10: Preparation of inhibitory factors and peptides as HA fusion proteins (such as HA-antiviral, HA-antibiotic, HA-enzyme inhibitor and HA-anti-allergic proteins)***

The cDNA for the peptide of interest such as an antibiotic peptide can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The peptide cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA , or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

***Example 11: Preparation of targeted HA fusion proteins***

The cDNA for the protein of interest can be isolated from cDNA library or can be made synthetically using several overlapping oligonucleotides using standard molecular

biology methods. The appropriate nucleotides can be engineered in the cDNA to form convenient restriction sites and also allow the attachment of the protein cDNA to albumin cDNA similar to the method described for hGH. Also a targeting protein or peptide cDNA such as single chain antibody or peptides, such as nuclear localization signals, that can direct proteins inside the cells can be fused to the other end of albumin. The protein of interest and the targeting peptide is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA, or pC4:HSA which allows the fusion with albumin cDNA. In this manner both N- and C-terminal end of albumin are fused to other proteins. The fused cDNA is then excised from pPPC0005 and is inserted into a plasmid such as pSAC35 to allow the expression of the albumin fusion protein in yeast. All the above procedures can be performed using standard methods in molecular biology. The albumin fusion protein secreted from yeast can be collected and purified from the media and tested for its biological activity and its targeting activity using appropriate biochemical and biological tests.

#### ***Example 12: Preparation of HA-enzymes fusions***

The cDNA for the enzyme of interest can be isolated by a variety of means including but not exclusively, from cDNA libraries, by RT-PCR and by PCR using a series of overlapping synthetic oligonucleotide primers, all using standard methods. The cDNA can be tailored at the 5' and 3' ends to generate restriction sites, such that oligonucleotide linkers can be used, for cloning of the cDNA into a vector containing the cDNA for HA. This can be at the N or C-terminus with or without the use of a spacer sequence. The enzyme cDNA is cloned into a vector such as pPPC0005 (Figure 4), pScCHSA, pScNHSA, or pC4:HSA from which the complete expression cassette is then excised and inserted into the plasmid pSAC35 to allow the expression of the albumin fusion protein in yeast. The albumin fusion protein secreted from the yeast can then be collected and purified from the media and tested for its biological activity. For expression in mammalian cell lines a similar procedure is adopted except that the expression cassette used employs a mammalian promoter, leader sequence and terminator (See Example 2). This expression cassette is then excised and inserted into a plasmid suitable for the transfection of mammalian cell lines.

### Example 13: Bacterial Expression of an Albumin Fusion Protein

A polynucleotide encoding an albumin fusion protein of the present invention comprising a bacterial signal sequence is amplified using PCR oligonucleotide primers corresponding to the 5' and 3' ends of the DNA sequence, to synthesize insertion fragments. The primers used to amplify the polynucleotide encoding insert should preferably contain restriction sites, such as BamHI and XbaI, at the 5' end of the primers in order to clone the amplified product into the expression vector. For example, BamHI and XbaI correspond to the restriction enzyme sites on the bacterial expression vector pQE-9. (Qiagen, Inc., Chatsworth, CA). This plasmid vector encodes antibiotic resistance (Amp<sup>r</sup>), a bacterial origin of replication (ori), an IPTG-regulatable promoter/operator (P/O), a ribosome binding site (RBS), a 6-histidine tag (6-His), and restriction enzyme cloning sites.

The pQE-9 vector is digested with BamHI and XbaI and the amplified fragment is ligated into the pQE-9 vector maintaining the reading frame initiated at the bacterial RBS. The ligation mixture is then used to transform the E. coli strain M15/rep4 (Qiagen, Inc.) which contains multiple copies of the plasmid pREP4, which expresses the lacI repressor and also confers kanamycin resistance (Kan<sup>r</sup>). Transformants are identified by their ability to grow on LB plates and ampicillin/kanamycin resistant colonies are selected. Plasmid DNA is isolated and confirmed by restriction analysis.

Clones containing the desired constructs are grown overnight (O/N) in liquid culture in LB media supplemented with both Amp (100 ug/ml) and Kan (25 ug/ml). The O/N culture is used to inoculate a large culture at a ratio of 1:100 to 1:250. The cells are grown to an optical density 600 (O.D.<sup>600</sup>) of between 0.4 and 0.6. IPTG (Isopropyl-B-D-thiogalacto pyranoside) is then added to a final concentration of 1 mM. IPTG induces by inactivating the lacI repressor, clearing the P/O leading to increased gene expression.

Cells are grown for an extra 3 to 4 hours. Cells are then harvested by centrifugation (20 mins at 6000Xg). The cell pellet is solubilized in the chaotropic agent 6 Molar Guanidine HCl or preferably in 8 M urea and concentrations greater than 0.14 M 2-mercaptoethanol by stirring for 3-4 hours at 4°C (see, e.g., Burton et al., Eur. J. Biochem. 179:379-387 (1989)). The cell debris is removed by centrifugation, and the supernatant containing the polypeptide is loaded onto a nickel-nitrilo-tri-acetic acid ("Ni-NTA")

affinity resin column (available from QIAGEN, Inc., *supra*). Proteins with a 6 x His tag bind to the Ni-NTA resin with high affinity and can be purified in a simple one-step procedure (for details see: The QIAexpressionist (1995) QIAGEN, Inc., *supra*).

Briefly, the supernatant is loaded onto the column in 6 M guanidine-HCl, pH 8.  
5 The column is first washed with 10 volumes of 6 M guanidine-HCl, pH 8, then washed with 10 volumes of 6 M guanidine-HCl pH 6, and finally the polypeptide is eluted with 6 M guanidine-HCl, pH 5.

The purified protein is then renatured by dialyzing it against phosphate-buffered saline (PBS) or 50 mM Na-acetate, pH 6 buffer plus 200 mM NaCl. Alternatively, the  
10 protein can be successfully refolded while immobilized on the Ni-NTA column. Exemplary conditions are as follows: renature using a linear 6M-1M urea gradient in 500 mM NaCl, 20% glycerol, 20 mM Tris/HCl pH 7.4, containing protease inhibitors. The renaturation should be performed over a period of 1.5 hours or more. After renaturation the proteins are eluted by the addition of 250 mM imidazole. Imidazole is removed by  
15 a final dialyzing step against PBS or 50 mM sodium acetate pH 6 buffer plus 200 mM NaCl. The purified protein is stored at 4° C or frozen at -80° C.

In addition to the above expression vector, the present invention further includes an expression vector, called pHE4a (ATCC Accession Number 209645, deposited on February 25, 1998) which contains phage operator and promoter elements operatively  
20 linked to a polynucleotide encoding an albumin fusion protein of the present invention, called pHE4a. (ATCC Accession Number 209645, deposited on February 25, 1998.) This vector contains: 1) a neomycinphosphotransferase gene as a selection marker, 2) an E. coli origin of replication, 3) a T5 phage promoter sequence, 4) two lac operator sequences, 5) a Shine-Delgarno sequence, and 6) the lactose operon repressor gene (lacIq). The origin of  
25 replication (oriC) is derived from pUC19 (LTI, Gaithersburg, MD). The promoter and operator sequences are made synthetically.

DNA can be inserted into the pHE4a by restricting the vector with NdeI and XbaI, BamHI, XhoI, or Asp718, running the restricted product on a gel, and isolating the larger fragment (the stuffer fragment should be about 310 base pairs). The DNA insert is  
30 generated according to PCR protocols described herein or otherwise known in the art, using PCR primers having restriction sites for NdeI (5' primer) and XbaI, BamHI, XhoI, or Asp718 (3' primer). The PCR insert is gel purified and restricted with compatible

enzymes. The insert and vector are ligated according to standard protocols.

The engineered vector may be substituted in the above protocol to express protein in a bacterial system.

#### 5           **Example 14: Expression of an Albumin Fusion Protein in Mammalian Cells**

The albumin fusion proteins of the present invention can be expressed in a mammalian cell. A typical mammalian expression vector contains a promoter element, which mediates the initiation of transcription of mRNA, a protein coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript.  
10 Additional elements include enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. Highly efficient transcription is achieved with the early and late promoters from SV40, the long terminal repeats (LTRs) from Retroviruses, e.g., RSV, HTLV, HIV and the early promoter of the cytomegalovirus (CMV). However, cellular elements can also be used (e.g., the human actin promoter).

15           Suitable expression vectors for use in practicing the present invention include, for example, vectors such as, pSVL and pMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146), pBC12MI (ATCC 67109), pCMVSPORT 2.0, and pCMVSPORT 3.0. Mammalian host cells that could be used include, but are not limited to, human HeLa, 293, H9 and Jurkat cells, mouse NIH3T3 and C127 cells, Cos 1, Cos 7  
20 and CV1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

Alternatively, the albumin fusion protein can be expressed in stable cell lines containing the polynucleotide encoding the albumin fusion protein integrated into a chromosome. The co-transfection with a selectable marker such as DHFR, gpt, neomycin, or hygromycin allows the identification and isolation of the transfected cells.

25           The transfected polynucleotide encoding the fusion protein can also be amplified to express large amounts of the encoded fusion protein. The DHFR (dihydrofolate reductase) marker is useful in developing cell lines that carry several hundred or even several thousand copies of the gene of interest. (See, e.g., Alt et al., J. Biol. Chem. 253:1357-1370 (1978); Hamlin et al., Biochem. et Biophys. Acta, 1097:107-143 (1990); Page et al.,  
30 Biotechnology 9:64-68 (1991)). Another useful selection marker is the enzyme glutamine synthase (GS) (Murphy et al., Biochem J. 227:277-279 (1991); Bebbington et al., Bio/Technology 10:169-175 (1992). Using these markers, the mammalian cells are grown

in selective medium and the cells with the highest resistance are selected. These cell lines contain the amplified gene(s) integrated into a chromosome. Chinese hamster ovary (CHO) and NSO cells are often used for the production of proteins.

Derivatives of the plasmid pSV2-dhfr (ATCC Accession No. 37146), the  
5 expression vectors pC4 (ATCC Accession No. 209646) and pC6 (ATCC Accession  
No.209647) contain the strong promoter (LTR) of the Rous Sarcoma Virus (Cullen et al.,  
Molecular and Cellular Biology, 438-447 (March, 1985)) plus a fragment of the CMV-  
enhancer (Boshart et al., Cell 41:521-530 (1985)). Multiple cloning sites, e.g., with the  
restriction enzyme cleavage sites BamHI, XbaI and Asp718, facilitate the cloning of the  
10 gene of interest. The vectors also contain the 3' intron, the polyadenylation and  
termination signal of the rat preproinsulin gene, and the mouse DHFR gene under control  
of the SV40 early promoter.

Specifically, the plasmid pC6, for example, is digested with appropriate restriction  
enzymes and then dephosphorylated using calf intestinal phosphates by procedures known  
15 in the art. The vector is then isolated from a 1% agarose gel.

A polynucleotide encoding an albumin fusion protein of the present invention is  
generated using techniques known in the art and this polynucleotide is amplified using  
PCR technology known in the art. If a naturally occurring signal sequence is used to  
produce the fusion protein of the present invention, the vector does not need a second  
20 signal peptide. Alternatively, if a naturally occurring signal sequence is not used, the  
vector can be modified to include a heterologous signal sequence. (See, e.g., International  
Publication No. WO 96/34891.)

The amplified fragment encoding the fusion protein of the invention is isolated  
from a 1% agarose gel using a commercially available kit ("Geneclean," BIO 101 Inc., La  
25 Jolla, Ca.). The fragment then is digested with appropriate restriction enzymes and again  
purified on a 1% agarose gel.

The amplified fragment encoding the albumin fusion protein of the invention is  
then digested with the same restriction enzyme and purified on a 1% agarose gel. The  
isolated fragment and the dephosphorylated vector are then ligated with T4 DNA ligase.  
30 *E. coli* HB101 or XL-1 Blue cells are then transformed and bacteria are identified that  
contain the fragment inserted into plasmid pC6 using, for instance, restriction enzyme  
analysis.



Chinese hamster ovary cells lacking an active DHFR gene is used for transfection. Five  $\mu\text{g}$  of the expression plasmid pC6 or pC4 is cotransfected with 0.5  $\mu\text{g}$  of the plasmid pSVneo using lipofectin (Felgner et al., *supra*). The plasmid pSV2-neo contains a dominant selectable marker, the *neo* gene from Tn5 encoding an enzyme that confers resistance to a group of antibiotics including G418. The cells are seeded in alpha minus MEM supplemented with 1 mg/ml G418. After 2 days, the cells are trypsinized and seeded in hybridoma cloning plates (Greiner, Germany) in alpha minus MEM supplemented with 10, 25, or 50 ng/ml of methotrexate plus 1 mg/ml G418. After about 10-14 days single clones are trypsinized and then seeded in 6-well petri dishes or 10 ml flasks using different concentrations of methotrexate (50 nM, 100 nM, 200 nM, 400 nM, 800 nM). Clones growing at the highest concentrations of methotrexate are then transferred to new 6-well plates containing even higher concentrations of methotrexate (1  $\mu\text{M}$ , 2  $\mu\text{M}$ , 5  $\mu\text{M}$ , 10 mM, 20 mM). The same procedure is repeated until clones are obtained which grow at a concentration of 100 - 200  $\mu\text{M}$ . Expression of the desired fusion protein is analyzed, for instance, by SDS-PAGE and Western blot or by reversed phase HPLC analysis.

#### Example 15: Multifusion Fusions

The albumin fusion proteins (e.g., containing a Therapeutic protein (or fragment or variant thereof) fused to albumin (or a fragment or variant thereof)) may additionally be fused to other proteins to generate "multifusion proteins". These multifusion proteins can be used for a variety of applications. For example, fusion of the albumin fusion proteins of the invention to His-tag, HA-tag, protein A, IgG domains, and maltose binding protein facilitates purification. (See e.g., EP A 394,827; Traunecker et al., Nature 331:84-86 (1988)). Nuclear localization signals fused to the polypeptides of the present invention can target the protein to a specific subcellular localization, while covalent heterodimer or homodimers can increase or decrease the activity of an albumin fusion protein. Furthermore, the fusion of additional protein sequences to the albumin fusion proteins of the invention may further increase the solubility and/or stability of the fusion protein. The fusion proteins described above can be made using or routinely modifying techniques known in the art and/or by modifying the following protocol, which outlines the fusion of a polypeptide to an IgG molecule.

Briefly, the human Fc portion of the IgG molecule can be PCR amplified, using primers that span the 5' and 3' ends of the sequence described below. These primers also should have convenient restriction enzyme sites that will facilitate cloning into an expression vector, preferably a mammalian or yeast expression vector.

5 For example, if pC4 (ATCC Accession No. 209646) is used, the human Fc portion can be ligated into the BamHI cloning site. Note that the 3' BamHI site should be destroyed. Next, the vector containing the human Fc portion is re-restricted with BamHI, linearizing the vector, and a polynucleotide encoding an albumin fusion protein of the present invention (generated and isolated using techniques known in the art), is ligated  
10 into this BamHI site. Note that the polynucleotide encoding the fusion protein of the invention is cloned without a stop codon, otherwise a Fc containing fusion protein will not be produced.

If the naturally occurring signal sequence is used to produce the albumin fusion protein of the present invention, pC4 does not need a second signal peptide. Alternatively,  
15 if the naturally occurring signal sequence is not used, the vector can be modified to include a heterologous signal sequence. (See, e.g., International Publication No. WO 96/34891.)

Human IgG Fc region:

GGGATCCGGAGCCCAAATCTTCTGACAAAACCTCACACATGCCACCGT  
20 GCCCAGCACCTGAATTCGAGGGTGCACCGTCAGTCTTCCTCTTCCCCCAAAA  
CCCAAGGACACCCTCATGATCTCCCGGACTCCTGAGGTACATGCGTGGTGGT  
GGACGTAAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGC  
GTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCA  
CGTACCGTGTGGTCAGCGTCCTACCGTCCTGCACCAGGACTGGCTGAATGGC  
25 AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAACCCCCATCGAGA  
AAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCCT  
GCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTG  
GTCAAAGGCTTCTATCCAAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGC  
AGCCGGAGAACAACACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC  
30 CTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGG  
AACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCA  
GAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGTGCGACGGCCGCGACTCTAGA

**Example 16: Production of an Antibody from an Albumin Fusion Protein****a) Hybridoma Technology**

5       Antibodies that bind the albumin fusion proteins of the present invention and portions of the albumin fusion proteins of the present invention (e.g., the Therapeutic protein portion or albumin portion of the fusion protein) can be prepared by a variety of methods. (See, Current Protocols, Chapter 2.) As one example of such methods, a preparation of an albumin fusion protein of the invention or a portion of an albumin fusion  
10       protein of the invention is prepared and purified to render it substantially free of natural contaminants. Such a preparation is then introduced into an animal in order to produce polyclonal antisera of greater specific activity.

Monoclonal antibodies specific for an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention, are prepared using hybridoma  
15       technology (Kohler et al., Nature 256:495 (1975); Kohler et al., Eur. J. Immunol. 6:511 (1976); Kohler et al., Eur. J. Immunol. 6:292 (1976); Hammerling et al., in: Monoclonal Antibodies and T-Cell Hybridomas, Elsevier, N.Y., pp. 563-681 (1981)). In general, an animal (preferably a mouse) is immunized with an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention. The splenocytes of such mice  
20       are extracted and fused with a suitable myeloma cell line. Any suitable myeloma cell line may be employed in accordance with the present invention; however, it is preferable to employ the parent myeloma cell line (SP2O), available from the ATCC. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium, and then cloned by limiting dilution as described by Wands et al. (Gastroenterology 80:225-232 (1981)). The  
25       hybridoma cells obtained through such a selection are then assayed to identify clones which secrete antibodies capable of binding an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention.

Alternatively, additional antibodies capable of binding to an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention can be  
30       produced in a two-step procedure using anti-idiotypic antibodies. Such a method makes use of the fact that antibodies are themselves antigens, and therefore, it is possible to obtain an antibody which binds to a second antibody. In accordance with this method,

protein specific antibodies are used to immunize an animal, preferably a mouse. The splenocytes of such an animal are then used to produce hybridoma cells, and the hybridoma cells are screened to identify clones which produce an antibody whose ability to bind to the an albumin fusion protein of the invention (or portion of an albumin fusion protein of the invention) -specific antibody can be blocked by the fusion protein of the invention, or a portion of an albumin fusion protein of the invention. Such antibodies comprise anti-idiotypic antibodies to the fusion protein of the invention (or portion of an albumin fusion protein of the invention) -specific antibody and are used to immunize an animal to induce formation of further fusion protein of the invention (or portion of an albumin fusion protein of the invention) -specific antibodies.

For *in vivo* use of antibodies in humans, an antibody is "humanized". Such antibodies can be produced using genetic constructs derived from hybridoma cells producing the monoclonal antibodies described above. Methods for producing chimeric and humanized antibodies are known in the art and are discussed herein. (See, for review, Morrison, Science 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Cabilly et al., U.S. Patent No. 4,816,567; Taniguchi et al., EP 171496; Morrison et al., EP 173494; Neuberger et al., WO 8601533; Robinson et al., International Publication No. WO 8702671; Boulianne et al., Nature 312:643 (1984); Neuberger et al., Nature 314:268 (1985)).

b) Isolation Of Antibody Fragments Directed Against an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention From A Library Of scFvs

Naturally occurring V-genes isolated from human PBLs are constructed into a library of antibody fragments which contain reactivities against an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention, to which the donor may or may not have been exposed (see e.g., U.S. Patent 5,885,793 incorporated herein by reference in its entirety).

*Rescue of the Library.* A library of scFvs is constructed from the RNA of human PBLs as described in International Publication No. WO 92/01047. To rescue phage displaying antibody fragments, approximately  $10^9$  *E. coli* harboring the phagemid are used to inoculate 50 ml of 2xTY containing 1% glucose and 100 µg/ml of ampicillin (2xTY-

AMP-GLU) and grown to an O.D. of 0.8 with shaking. Five ml of this culture is used to inoculate 50 ml of 2xTY-AMP-GLU, 2 x 10<sup>8</sup> TU of delta gene 3 helper (M13 delta gene III, see International Publication No. WO 92/01047) are added and the culture incubated at 37°C for 45 minutes without shaking and then at 37°C for 45 minutes with shaking. The culture is centrifuged at 4000 r.p.m. for 10 min. and the pellet resuspended in 2 liters of 2xTY containing 100 µg/ml ampicillin and 50 µg/ml kanamycin and grown overnight. Phage are prepared as described in International Publication No. WO 92/01047.

M13 delta gene III is prepared as follows: M13 delta gene III helper phage does not encode gene III protein, hence the phage(mid) displaying antibody fragments have a greater avidity of binding to antigen. Infectious M13 delta gene III particles are made by growing the helper phage in cells harboring a pUC19 derivative supplying the wild type gene III protein during phage morphogenesis. The culture is incubated for 1 hour at 37°C without shaking and then for a further hour at 37°C with shaking. Cells are spun down (IEC-Centra 8,400 r.p.m. for 10 min), resuspended in 300 ml 2xTY broth containing 100 µg ampicillin/ml and 25 µg kanamycin/ml (2xTY-AMP-KAN) and grown overnight, shaking at 37°C. Phage particles are purified and concentrated from the culture medium by two PEG-precipitations (Sambrook et al., 1990), resuspended in 2 ml PBS and passed through a 0.45 µm filter (Minisart NML; Sartorius) to give a final concentration of approximately 10<sup>13</sup> transducing units/ml (ampicillin-resistant clones).

*Panning of the Library.* Immuntubes (Nunc) are coated overnight in PBS with 4 ml of either 100 µg/ml or 10 µg/ml of an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention. Tubes are blocked with 2% Marvel-PBS for 2 hours at 37°C and then washed 3 times in PBS. Approximately 10<sup>13</sup> TU of phage is applied to the tube and incubated for 30 minutes at room temperature tumbling on an over and under turntable and then left to stand for another 1.5 hours. Tubes are washed 10 times with PBS 0.1% Tween-20 and 10 times with PBS. Phage are eluted by adding 1 ml of 100 mM triethylamine and rotating 15 minutes on an under and over turntable after which the solution is immediately neutralized with 0.5 ml of 1.0M Tris-HCl, pH 7.4. Phage are then used to infect 10 ml of mid-log E. coli TG1 by incubating eluted phage with bacteria for 30 minutes at 37°C. The E. coli are then plated on TYE plates containing 1% glucose and 100 µg/ml ampicillin. The resulting bacterial library is then rescued with delta gene 3 helper phage as described above to prepare phage for a subsequent round of

selection. This process is then repeated for a total of 4 rounds of affinity purification with tube-washing increased to 20 times with PBS, 0.1% Tween-20 and 20 times with PBS for rounds 3 and 4.

5      *Characterization of Binders.* Eluted phage from the 3rd and 4th rounds of selection are used to infect E. coli HB 2151 and soluble scFv is produced (Marks, et al., 1991) from single colonies for assay. ELISAs are performed with microtitre plates coated with either 10 pg/ml of an albumin fusion protein of the invention, or a portion of an albumin fusion protein of the invention, in 50 mM bicarbonate pH 9.6. Clones positive in ELISA are further characterized by PCR fingerprinting (see, e.g., International Publication  
10 No. WO 92/01047) and then by sequencing. These ELISA positive clones may also be further characterized by techniques known in the art, such as, for example, epitope mapping, binding affinity, receptor signal transduction, ability to block or competitively inhibit antibody/antigen binding, and competitive agonistic or antagonistic activity.

#### 15      **Example 17: Method of Treatment Using Gene Therapy-Ex Vivo**

One method of gene therapy transplants fibroblasts, which are capable of expressing an albumin fusion protein of the present invention, onto a patient. Generally, fibroblasts are obtained from a subject by skin biopsy. The resulting tissue is placed in  
20 tissue-culture medium and separated into small pieces. Small chunks of the tissue are placed on a wet surface of a tissue culture flask; approximately ten pieces are placed in each flask. The flask is turned upside down, closed tight and left at room temperature over night. After 24 hours at room temperature, the flask is inverted and the chunks of tissue remain fixed to the bottom of the flask and fresh media (e.g., Ham's F12 media, with 10%  
25 FBS, penicillin and streptomycin) is added. The flasks are then incubated at 37 degree C for approximately one week.

At this time, fresh media is added and subsequently changed every several days. After an additional two weeks in culture, a monolayer of fibroblasts emerge. The monolayer is trypsinized and scaled into larger flasks.

30      pMV-7 (Kirschmeier, P.T. et al., DNA, 7:219-25 (1988)), flanked by the long terminal repeats of the Moloney murine sarcoma virus, is digested with EcoRI and HindIII and subsequently treated with calf intestinal phosphatase. The linear vector is fractionated

on agarose gel and purified, using glass beads.

Polynucleotides encoding an albumin fusion protein of the invention can be generated using techniques known in the art amplified using PCR primers which correspond to the 5' and 3' end sequences and optionally having appropriate restriction sites and initiation/stop codons, if necessary. Preferably, the 5' primer contains an EcoRI site and the 3' primer includes a HindIII site. Equal quantities of the Moloney murine sarcoma virus linear backbone and the amplified EcoRI and HindIII fragment are added together, in the presence of T4 DNA ligase. The resulting mixture is maintained under conditions appropriate for ligation of the two fragments. The ligation mixture is then used to transform bacteria HB101, which are then plated onto agar containing kanamycin for the purpose of confirming that the vector has the gene of interest properly inserted.

The amphotropic pA317 or GP+am12 packaging cells are grown in tissue culture to confluent density in Dulbecco's Modified Eagles Medium (DMEM) with 10% calf serum (CS), penicillin and streptomycin. The MSV vector containing the gene is then added to the media and the packaging cells transduced with the vector. The packaging cells now produce infectious viral particles containing the gene (the packaging cells are now referred to as producer cells).

Fresh media is added to the transduced producer cells, and subsequently, the media is harvested from a 10 cm plate of confluent producer cells. The spent media, containing the infectious viral particles, is filtered through a millipore filter to remove detached producer cells and this media is then used to infect fibroblast cells. Media is removed from a sub-confluent plate of fibroblasts and quickly replaced with the media from the producer cells. This media is removed and replaced with fresh media. If the titer of virus is high, then virtually all fibroblasts will be infected and no selection is required. If the titer is very low, then it is necessary to use a retroviral vector that has a selectable marker, such as neo or his. Once the fibroblasts have been efficiently infected, the fibroblasts are analyzed to determine whether the albumin fusion protein is produced.

The engineered fibroblasts are then transplanted onto the host, either alone or after having been grown to confluence on cytodex 3 microcarrier beads.

#### **Example 18: Method of Treatment Using Gene Therapy - In Vivo**

Another aspect of the present invention is using *in vivo* gene therapy methods to

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treat disorders, diseases and conditions. The gene therapy method relates to the introduction of naked nucleic acid (DNA, RNA, and antisense DNA or RNA) sequences encoding an albumin fusion protein of the invention into an animal. Polynucleotides encoding albumin fusion proteins of the present invention may be operatively linked to  
5 (i.e., associated with) a promoter or any other genetic elements necessary for the expression of the polypeptide by the target tissue. Such gene therapy and delivery techniques and methods are known in the art, see, for example, WO90/11092, WO98/11779; U.S. Patent NO. 5693622, 5705151, 5580859; Tabata et al., *Cardiovasc. Res.* 35(3):470-479 (1997); Chao et al., *Pharmacol. Res.* 35(6):517-522 (1997); Wolff,  
10 *Neuromuscul. Disord.* 7(5):314-318 (1997); Schwartz et al., *Gene Ther.* 3(5):405-411 (1996); Tsurumi et al., *Circulation* 94(12):3281-3290 (1996) (incorporated herein by reference).

The polynucleotide constructs may be delivered by any method that delivers injectable materials to the cells of an animal, such as, injection into the interstitial space of  
15 tissues (heart, muscle, skin, lung, liver, intestine and the like). The polynucleotide constructs can be delivered in a pharmaceutically acceptable liquid or aqueous carrier.

The term "naked" polynucleotide, DNA or RNA, refers to sequences that are free from any delivery vehicle that acts to assist, promote, or facilitate entry into the cell, including viral sequences, viral particles, liposome formulations, lipofectin or precipitating  
20 agents and the like. However, polynucleotides encoding albumin fusion proteins of the present invention may also be delivered in liposome formulations (such as those taught in Felgner P.L. et al. (1995) *Ann. NY Acad. Sci.* 772:126-139 and Abdallah B. et al. (1995) *Biol. Cell* 85(1):1-7) which can be prepared by methods well known to those skilled in the art.

25 The polynucleotide vector constructs used in the gene therapy method are preferably constructs that will not integrate into the host genome nor will they contain sequences that allow for replication. Any strong promoter known to those skilled in the art can be used for driving the expression of DNA. Unlike other gene therapy techniques, one major advantage of introducing naked nucleic acid sequences into target cells is the  
30 transitory nature of the polynucleotide synthesis in the cells. Studies have shown that non-replicating DNA sequences can be introduced into cells to provide production of the desired polypeptide for periods of up to six months.



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The polynucleotide construct can be delivered to the interstitial space of tissues within an animal, including muscle, skin, brain, lung, liver, spleen, bone marrow, thymus, heart, lymph, blood, bone, cartilage, pancreas, kidney, gall bladder, stomach, intestine, testis, ovary, uterus, rectum, nervous system, eye, gland, and connective tissue. Interstitial space of the tissues comprises the intercellular fluid, mucopolysaccharide matrix among the reticular fibers of organ tissues, elastic fibers in the walls of vessels or chambers, collagen fibers of fibrous tissues, or that same matrix within connective tissue ensheathing muscle cells or in the lacunae of bone. It is similarly the space occupied by the plasma of the circulation and the lymph fluid of the lymphatic channels. Delivery to the interstitial space of muscle tissue is preferred for the reasons discussed below. They may be conveniently delivered by injection into the tissues comprising these cells. They are preferably delivered to and expressed in persistent, non-dividing cells which are differentiated, although delivery and expression may be achieved in non-differentiated or less completely differentiated cells, such as, for example, stem cells of blood or skin fibroblasts. *In vivo* muscle cells are particularly competent in their ability to take up and express polynucleotides.

For the naked polynucleotide injection, an effective dosage amount of DNA or RNA will be in the range of from about 0.05 g/kg body weight to about 50 mg/kg body weight. Preferably the dosage will be from about 0.005 mg/kg to about 20 mg/kg and more preferably from about 0.05 mg/kg to about 5 mg/kg. Of course, as the artisan of ordinary skill will appreciate, this dosage will vary according to the tissue site of injection. The appropriate and effective dosage of nucleic acid sequence can readily be determined by those of ordinary skill in the art and may depend on the condition being treated and the route of administration. The preferred route of administration is by the parenteral route of injection into the interstitial space of tissues. However, other parenteral routes may also be used, such as, inhalation of an aerosol formulation particularly for delivery to lungs or bronchial tissues, throat or mucous membranes of the nose. In addition, naked polynucleotide constructs can be delivered to arteries during angioplasty by the catheter used in the procedure.

The dose response effects of injected polynucleotide in muscle *in vivo* is determined as follows. Suitable template DNA for production of mRNA coding for polypeptide of the present invention is prepared in accordance with a standard

recombinant DNA methodology. The template DNA, which may be either circular or linear, is either used as naked DNA or complexed with liposomes. The quadriceps muscles of mice are then injected with various amounts of the template DNA.

Five to six week old female and male Balb/C mice are anesthetized by intraperitoneal injection with 0.3 ml of 2.5% Avertin. A 1.5 cm incision is made on the anterior thigh, and the quadriceps muscle is directly visualized. The template DNA is injected in 0.1 ml of carrier in a 1 cc syringe through a 27 gauge needle over one minute, approximately 0.5 cm from the distal insertion site of the muscle into the knee and about 0.2 cm deep. A suture is placed over the injection site for future localization, and the skin is closed with stainless steel clips.

After an appropriate incubation time (e.g., 7 days) muscle extracts are prepared by excising the entire quadriceps. Every fifth 15 um cross-section of the individual quadriceps muscles is histochemically stained for protein expression. A time course for fusion protein expression may be done in a similar fashion except that quadriceps from different mice are harvested at different times. Persistence of DNA in muscle following injection may be determined by Southern blot analysis after preparing total cellular DNA and HIRT supernatants from injected and control mice. The results of the above experimentation in mice can be used to extrapolate proper dosages and other treatment parameters in humans and other animals using naked DNA.

#### **Example 19: Transgenic Animals**

The albumin fusion proteins of the invention can also be expressed in transgenic animals. Animals of any species, including, but not limited to, mice, rats, rabbits, hamsters, guinea pigs, pigs, micro-pigs, goats, sheep, cows and non-human primates, e.g., baboons, monkeys, and chimpanzees may be used to generate transgenic animals. In a specific embodiment, techniques described herein or otherwise known in the art, are used to express fusion proteins of the invention in humans, as part of a gene therapy protocol.

Any technique known in the art may be used to introduce the polynucleotides encoding the albumin fusion proteins of the invention into animals to produce the founder lines of transgenic animals. Such techniques include, but are not limited to, pronuclear microinjection (Paterson et al., Appl. Microbiol. Biotechnol. 40:691-698 (1994); Carver et al., Biotechnology (NY) 11:1263-1270 (1993); Wright et al., Biotechnology (NY) 9:830-

834 (1991); and Hoppe et al., U.S. Pat. No. 4,873,191 (1989)); retrovirus mediated gene transfer into germ lines (Van der Putten et al., Proc. Natl. Acad. Sci., USA 82:6148-6152 (1985)), blastocysts or embryos; gene targeting in embryonic stem cells (Thompson et al., Cell 56:313-321 (1989)); electroporation of cells or embryos (Lo, 1983, Mol Cell. Biol. 3:1803-1814 (1983)); introduction of the polynucleotides of the invention using a gene gun (see, e.g., Ulmer et al., Science 259:1745 (1993)); introducing nucleic acid constructs into embryonic pluripotent stem cells and transferring the stem cells back into the blastocyst; and sperm-mediated gene transfer (Lavitrano et al., Cell 57:717-723 (1989); etc. For a review of such techniques, see Gordon, "Transgenic Animals," Intl. Rev. Cytol. 115:171-229 (1989), which is incorporated by reference herein in its entirety.

Any technique known in the art may be used to produce transgenic clones containing polynucleotides encoding albumin fusion proteins of the invention, for example, nuclear transfer into enucleated oocytes of nuclei from cultured embryonic, fetal, or adult cells induced to quiescence (Campell et al., Nature 380:64-66 (1996); Wilmut et al., Nature 385:810-813 (1997)).

The present invention provides for transgenic animals that carry the polynucleotides encoding the albumin fusion proteins of the invention in all their cells, as well as animals which carry these polynucleotides in some, but not all their cells, *i.e.*, mosaic animals or chimeric. The transgene may be integrated as a single transgene or as multiple copies such as in concatamers, *e.g.*, head-to-head tandems or head-to-tail tandems. The transgene may also be selectively introduced into and activated in a particular cell type by following, for example, the teaching of Lasko et al. (Lasko et al., Proc. Natl. Acad. Sci. USA 89:6232-6236 (1992)). The regulatory sequences required for such a cell-type specific activation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art. When it is desired that the polynucleotide encoding the fusion protein of the invention be integrated into the chromosomal site of the endogenous gene corresponding to the Therapeutic protein portion or albumin portion of the fusion protein of the invention, gene targeting is preferred. Briefly, when such a technique is to be utilized, vectors containing some nucleotide sequences homologous to the endogenous gene are designed for the purpose of integrating, via homologous recombination with chromosomal sequences, into and disrupting the function of the nucleotide sequence of the endogenous gene. The transgene may also be selectively

introduced into a particular cell type, thus inactivating the endogenous gene in only that cell type, by following, for example, the teaching of Gu et al. (Gu et al., Science 265:103-106 (1994)). The regulatory sequences required for such a cell-type specific inactivation will depend upon the particular cell type of interest, and will be apparent to those of skill in the art.

Once transgenic animals have been generated, the expression of the recombinant gene may be assayed utilizing standard techniques. Initial screening may be accomplished by Southern blot analysis or PCR techniques to analyze animal tissues to verify that integration of the polynucleotide encoding the fusion protein of the invention has taken place. The level of mRNA expression of the polynucleotide encoding the fusion protein of the invention in the tissues of the transgenic animals may also be assessed using techniques which include, but are not limited to, Northern blot analysis of tissue samples obtained from the animal, *in situ* hybridization analysis, and reverse transcriptase-PCR (rt-PCR). Samples of fusion protein-expressing tissue may also be evaluated immunocytochemically or immunohistochemically using antibodies specific for the fusion protein.

Once the founder animals are produced, they may be bred, inbred, outbred, or crossbred to produce colonies of the particular animal. Examples of such breeding strategies include, but are not limited to: outbreeding of founder animals with more than one integration site in order to establish separate lines; inbreeding of separate lines in order to produce compound transgenics that express the transgene at higher levels because of the effects of additive expression of each transgene; crossing of heterozygous transgenic animals to produce animals homozygous for a given integration site in order to both augment expression and eliminate the need for screening of animals by DNA analysis; crossing of separate homozygous lines to produce compound heterozygous or homozygous lines; and breeding to place the transgene (i.e., polynucleotide encoding an albumin fusion protein of the invention) on a distinct background that is appropriate for an experimental model of interest.

Transgenic animals of the invention have uses which include, but are not limited to, animal model systems useful in elaborating the biological function of fusion proteins of the invention and the Therapeutic protein and/or albumin component of the fusion protein of the invention, studying conditions and/or disorders associated with aberrant expression,

and in screening for compounds effective in ameliorating such conditions and/or disorders.

**Example 20: Assays Detecting Stimulation or Inhibition of B cell Proliferation and Differentiation**

5        Generation of functional humoral immune responses requires both soluble and cognate signaling between B-lineage cells and their microenvironment. Signals may impart a positive stimulus that allows a B-lineage cell to continue its programmed development, or a negative stimulus that instructs the cell to arrest its current developmental pathway. To date, numerous stimulatory and inhibitory signals have been  
10       found to influence B cell responsiveness including IL-2, IL-4, IL-5, IL-6, IL-7, IL10, IL-13, IL-14 and IL-15. Interestingly, these signals are by themselves weak effectors but can, in combination with various co-stimulatory proteins, induce activation, proliferation, differentiation, homing, tolerance and death among B cell populations.

15       One of the best studied classes of B-cell co-stimulatory proteins is the TNF-superfamily. Within this family CD40, CD27, and CD30 along with their respective ligands CD154, CD70, and CD153 have been found to regulate a variety of immune responses. Assays which allow for the detection and/or observation of the proliferation and differentiation of these B-cell populations and their precursors are valuable tools in  
20       determining the effects various proteins may have on these B-cell populations in terms of proliferation and differentiation. Listed below are two assays designed to allow for the detection of the differentiation, proliferation, or inhibition of B-cell populations and their precursors.

25       In Vitro Assay- Albumin fusion proteins of the invention (including fusion proteins containing fragments or variants of Therapeutic proteins and/or albumin or fragments or variants of albumin) can be assessed for its ability to induce activation, proliferation, differentiation or inhibition and/or death in B-cell populations and their precursors. The activity of an albumin fusion protein of the invention on purified human tonsillar B cells, measured qualitatively over the dose range from 0.1 to 10,000 ng/mL, is assessed in a standard B-lymphocyte co-stimulation assay in which purified tonsillar B cells are cultured  
30       in the presence of either formalin-fixed *Staphylococcus aureus* Cowan I (SAC) or immobilized anti-human IgM antibody as the priming agent. Second signals such as IL-2 and IL-15 synergize with SAC and IgM crosslinking to elicit B cell proliferation as

measured by tritiated-thymidine incorporation. Novel synergizing agents can be readily identified using this assay. The assay involves isolating human tonsillar B cells by magnetic bead (MACS) depletion of CD3-positive cells. The resulting cell population is greater than 95% B cells as assessed by expression of CD45R(B220).

5        Various dilutions of each sample are placed into individual wells of a 96-well plate to which are added  $10^5$  B-cells suspended in culture medium (RPMI 1640 containing 10% FBS,  $5 \times 10^{-5}$ M 2ME, 100U/ml penicillin, 10ug/ml streptomycin, and  $10^{-5}$  dilution of SAC) in a total volume of 150ul. Proliferation or inhibition is quantitated by a 20h pulse (1uCi/well) with  $^3$ H-thymidine (6.7 Ci/mM) beginning 72h post factor addition. The  
10       positive and negative controls are IL2 and medium respectively.

*In vivo* Assay- BALB/c mice are injected (i.p.) twice per day with buffer only, or 2 mg/Kg of an albumin fusion protein of the invention (including fusion proteins containing fragments or variants of Therapeutic proteins and/or albumin or fragments or variants of albumin). Mice receive this treatment for 4 consecutive days, at which time they are  
15       sacrificed and various tissues and serum collected for analyses. Comparison of H&E sections from normal spleens and spleens treated with the albumin fusion protein of the invention identify the results of the activity of the fusion protein on spleen cells, such as the diffusion of peri-arterial lymphatic sheaths, and/or significant increases in the nucleated cellularity of the red pulp regions, which may indicate the activation of the  
20       differentiation and proliferation of B-cell populations. Immunohistochemical studies using a B cell marker, anti-CD45R(B220), are used to determine whether any physiological changes to splenic cells, such as splenic disorganization, are due to increased B-cell representation within loosely defined B-cell zones that infiltrate established T-cell regions.

      Flow cytometric analyses of the spleens from mice treated with the albumin fusion  
25       protein is used to indicate whether the albumin fusion protein specifically increases the proportion of ThB+, CD45R(B220)dull B cells over that which is observed in control mice.

      Likewise, a predicted consequence of increased mature B-cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgM and IgA levels are  
30       compared between buffer and fusion protein treated mice.

      The studies described in this example tested activity of fusion proteins of the invention. However, one skilled in the art could easily modify the exemplified studies to

test the activity of fusion proteins and polynucleotides of the invention (e.g., gene therapy).

#### **Example 21: T Cell Proliferation Assay**

5 A CD3-induced proliferation assay is performed on PBMCs and is measured by the uptake of  $^3\text{H}$ -thymidine. The assay is performed as follows. Ninety-six well plates are coated with 100  $\mu\text{l}$ /well of mAb to CD3 (HIT3a, Pharmingen) or isotype-matched control mAb (B33.1) overnight at 4 degrees C (1  $\mu\text{g}/\text{ml}$  in .05M bicarbonate buffer, pH 9.5), then washed three times with PBS. PBMC are isolated by F/H gradient centrifugation from  
10 human peripheral blood and added to quadruplicate wells ( $5 \times 10^4$ /well) of mAb coated plates in RPMI containing 10% FCS and P/S in the presence of varying concentrations of an albumin fusion protein of the invention (including fusion proteins containing fragments or variants of Therapeutic proteins and/or albumin or fragments or variants of albumin) (total volume 200  $\mu\text{l}$ ). Relevant protein buffer and medium alone are controls. After 48  
15 hr. culture at 37 degrees C, plates are spun for 2 min. at 1000 rpm and 100  $\mu\text{l}$  of supernatant is removed and stored -20 degrees C for measurement of IL-2 (or other cytokines) if effect on proliferation is observed. Wells are supplemented with 100  $\mu\text{l}$  of medium containing 0.5  $\mu\text{Ci}$  of  $^3\text{H}$ -thymidine and cultured at 37 degrees C for 18-24 hr. Wells are harvested and incorporation of  $^3\text{H}$ -thymidine used as a measure of proliferation.  
20 Anti-CD3 alone is the positive control for proliferation. IL-2 (100 U/ml) is also used as a control which enhances proliferation. Control antibody which does not induce proliferation of T cells is used as the negative control for the effects of fusion proteins of the invention.

The studies described in this example tested activity of fusion proteins of the  
25 invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of fusion proteins or polynucleotides of the invention (e.g., gene therapy).

#### **Example 22: Effect of Fusion Proteins of the Invention on the Expression of MHC Class II, Costimulatory and Adhesion Molecules and Cell Differentiation of 30 Monocytes and Monocyte-Derived Human Dendritic Cells**

Dendritic cells are generated by the expansion of proliferating precursors found in the peripheral blood: adherent PBMC or elutriated monocytic fractions are cultured for 7-

10 days with GM-CSF (50 ng/ml) and IL-4 (20 ng/ml). These dendritic cells have the characteristic phenotype of immature cells (expression of CD1, CD80, CD86, CD40 and MHC class II antigens). Treatment with activating factors, such as TNF- $\alpha$ , causes a rapid change in surface phenotype (increased expression of MHC class I and II, costimulatory and adhesion molecules, downregulation of FC $\gamma$ RII, upregulation of CD83). These changes correlate with increased antigen-presenting capacity and with functional maturation of the dendritic cells.

FACS analysis of surface antigens is performed as follows. Cells are treated 1-3 days with increasing concentrations of an albumin fusion protein of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

Effect on the production of cytokines. Cytokines generated by dendritic cells, in particular IL-12, are important in the initiation of T-cell dependent immune responses. IL-12 strongly influences the development of Th1 helper T-cell immune response, and induces cytotoxic T and NK cell function. An ELISA is used to measure the IL-12 release as follows. Dendritic cells ( $10^6$ /ml) are treated with increasing concentrations of an albumin fusion protein of the invention for 24 hours. LPS (100 ng/ml) is added to the cell culture as positive control. Supernatants from the cell cultures are then collected and analyzed for IL-12 content using commercial ELISA kit (e.g., R & D Systems (Minneapolis, MN)). The standard protocols provided with the kits are used.

Effect on the expression of MHC Class II, costimulatory and adhesion molecules. Three major families of cell surface antigens can be identified on monocytes: adhesion molecules, molecules involved in antigen presentation, and Fc receptor. Modulation of the expression of MHC class II antigens and other costimulatory molecules, such as B7 and ICAM-1, may result in changes in the antigen presenting capacity of monocytes and ability to induce T cell activation. Increased expression of Fc receptors may correlate with improved monocyte cytotoxic activity, cytokine release and phagocytosis.

FACS analysis is used to examine the surface antigens as follows. Monocytes are



5 treated 1-5 days with increasing concentrations of an albumin fusion protein of the invention or LPS (positive control), washed with PBS containing 1% BSA and 0.02 mM sodium azide, and then incubated with 1:20 dilution of appropriate FITC- or PE-labeled monoclonal antibodies for 30 minutes at 4 degrees C. After an additional wash, the labeled cells are analyzed by flow cytometry on a FACScan (Becton Dickinson).

10 Monocyte activation and/or increased survival. Assays for molecules that activate (or alternatively, inactivate) monocytes and/or increase monocyte survival (or alternatively, decrease monocyte survival) are known in the art and may routinely be applied to determine whether a molecule of the invention functions as an inhibitor or activator of monocytes. Albumin fusion proteins of the invention can be screened using the three assays described below. For each of these assays, Peripheral blood mononuclear cells (PBMC) are purified from single donor leukopacks (American Red Cross, Baltimore, MD) by centrifugation through a Histopaque gradient (Sigma). Monocytes are isolated from PBMC by counterflow centrifugal elutriation.

20 Monocyte Survival Assay. Human peripheral blood monocytes progressively lose viability when cultured in absence of serum or other stimuli. Their death results from internally regulated processes (apoptosis). Addition to the culture of activating factors, such as TNF-alpha dramatically improves cell survival and prevents DNA fragmentation. Propidium iodide (PI) staining is used to measure apoptosis as follows. Monocytes are cultured for 48 hours in polypropylene tubes in serum-free medium (positive control), in the presence of 100 ng/ml TNF-alpha (negative control), and in the presence of varying concentrations of the fusion protein to be tested. Cells are suspended at a concentration of 25  $2 \times 10^6$ /ml in PBS containing PI at a final concentration of 5  $\mu$ g/ml, and then incubated at room temperature for 5 minutes before FACScan -analysis. PI uptake has been demonstrated to correlate with DNA fragmentation in this experimental paradigm.

30 Effect on cytokine release. An important function of monocytes/macrophages is their regulatory activity on other cellular populations of the immune system through the release of cytokines after stimulation. An ELISA to measure cytokine release is performed as follows. Human monocytes are incubated at a density of  $5 \times 10^5$  cells/ml with increasing

concentrations of an albumin fusion protein of the invention and under the same conditions, but in the absence of the fusion protein. For IL-12 production, the cells are primed overnight with IFN (100 U/ml) in the presence of the fusion protein. LPS (10 ng/ml) is then added. Conditioned media are collected after 24h and kept frozen until use.

- 5 Measurement of TNF-alpha, IL-10, MCP-1 and IL-8 is then performed using a commercially available ELISA kit (e.g., R & D Systems (Minneapolis, MN)) and applying the standard protocols provided with the kit.

Oxidative burst. Purified monocytes are plated in 96-w plate at  $2 \times 10^5$  cell/well.

- 10 Increasing concentrations of an albumin fusion protein of the invention are added to the wells in a total volume of 0.2 ml culture medium (RPMI 1640 + 10% FCS, glutamine and antibiotics). After 3 days incubation, the plates are centrifuged and the medium is removed from the wells. To the macrophage monolayers, 0.2 ml per well of phenol red solution (140 mM NaCl, 10 mM potassium phosphate buffer pH 7.0, 5.5 mM dextrose, 0.56 mM
- 15 phenol red and 19 U/ml of HRPO) is added, together with the stimulant (200 nM PMA). The plates are incubated at 37°C for 2 hours and the reaction is stopped by adding 20  $\mu$ l 1N NaOH per well. The absorbance is read at 610 nm. To calculate the amount of  $H_2O_2$  produced by the macrophages, a standard curve of a  $H_2O_2$  solution of known molarity is performed for each experiment.

- 20 The studies described in this example tested activity of fusion proteins of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of fusion proteins or polynucleotides of the invention (e.g., gene therapy).

### **Example 23: Biological Effects of Fusion Proteins of the Invention**

- 25 Astrocyte and Neuronal Assays

Albumin fusion proteins of the invention can be tested for activity in promoting the survival, neurite outgrowth, or phenotypic differentiation of cortical neuronal cells and for inducing the proliferation of glial fibrillary acidic protein immunopositive cells, astrocytes.

- The selection of cortical cells for the bioassay is based on the prevalent expression of FGF-1 and FGF-2 in cortical structures and on the previously reported enhancement of
- 30 cortical neuronal survival resulting from FGF-2 treatment. A thymidine incorporation

assay, for example, can be used to elucidate an albumin fusion protein of the invention's activity on these cells.

Moreover, previous reports describing the biological effects of FGF-2 (basic FGF) on cortical or hippocampal neurons *in vitro* have demonstrated increases in both neuron survival and neurite outgrowth (Walicke et al., "Fibroblast growth factor promotes survival of dissociated hippocampal neurons and enhances neurite extension." *Proc. Natl. Acad. Sci. USA* 83:3012-3016. (1986), assay herein incorporated by reference in its entirety). However, reports from experiments done on PC-12 cells suggest that these two responses are not necessarily synonymous and may depend on not only which FGF is being tested but also on which receptor(s) are expressed on the target cells. Using the primary cortical neuronal culture paradigm, the ability of an albumin fusion protein of the invention to induce neurite outgrowth can be compared to the response achieved with FGF-2 using, for example, a thymidine incorporation assay.

#### Fibroblast and endothelial cell assays

Human lung fibroblasts are obtained from Clonetics (San Diego, CA) and maintained in growth media from Clonetics. Dermal microvascular endothelial cells are obtained from Cell Applications (San Diego, CA). For proliferation assays, the human lung fibroblasts and dermal microvascular endothelial cells can be cultured at 5,000 cells/well in a 96-well plate for one day in growth medium. The cells are then incubated for one day in 0.1% BSA basal medium. After replacing the medium with fresh 0.1% BSA medium, the cells are incubated with the test fusion protein of the invention proteins for 3 days. Alamar Blue (Alamar Biosciences, Sacramento, CA) is added to each well to a final concentration of 10%. The cells are incubated for 4 hr. Cell viability is measured by reading in a CytoFluor fluorescence reader. For the PGE<sub>2</sub> assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or fusion protein of the invention with or without IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for PGE<sub>2</sub> by ELA kit (Cayman, Ann Arbor, MI). For the IL-6 assays, the human lung fibroblasts are cultured at 5,000 cells/well in a 96-well plate for one day. After a

medium change to 0.1% BSA basal medium, the cells are incubated with FGF-2 or with or without an albumin fusion protein of the invention and/or IL-1 $\alpha$  for 24 hours. The supernatants are collected and assayed for IL-6 by ELISA kit (Endogen, Cambridge, MA).

Human lung fibroblasts are cultured with FGF-2 or an albumin fusion protein of the invention for 3 days in basal medium before the addition of Alamar Blue to assess effects on growth of the fibroblasts. FGF-2 should show a stimulation at 10 - 2500 ng/ml which can be used to compare stimulation with the fusion protein of the invention.

#### Cell proliferation based on [3H]thymidine incorporation

The following [3H]Thymidine incorporation assay can be used to measure the effect of a Therapeutic proteins, e.g., growth factor proteins, on the proliferation of cells such as fibroblast cells, epithelial cells or immature muscle cells.

Sub-confluent cultures are arrested in G1 phase by an 18 h incubation in serum-free medium. Therapeutic proteins are then added for 24 h and during the last 4 h, the cultures are labeled with [3H]thymidine, at a final concentration of 0.33  $\mu$ M (25 Ci/mmol, Amersham, Arlington Heights, IL). The incorporated [3H]thymidine is precipitated with ice-cold 10% trichloroacetic acid for 24 h. Subsequently, the cells are rinsed sequentially with ice-cold 10% trichloroacetic acid and then with ice-cold water. Following lysis in 0.5 M NaOH, the lysates and PBS rinses (500 ml) are pooled, and the amount of radioactivity is measured.

#### Parkinson Models.

The loss of motor function in Parkinson's disease is attributed to a deficiency of striatal dopamine resulting from the degeneration of the nigrostriatal dopaminergic projection neurons. An animal model for Parkinson's that has been extensively characterized involves the systemic administration of 1-methyl-4 phenyl 1,2,3,6-tetrahydropyridine (MPTP). In the CNS, MPTP is taken-up by astrocytes and catabolized by monoamine oxidase B to 1-methyl-4-phenyl pyridine (MPP<sup>+</sup>) and released. Subsequently, MPP<sup>+</sup> is actively accumulated in dopaminergic neurons by the high-affinity reuptake transporter for dopamine. MPP<sup>+</sup> is then concentrated in mitochondria by the electrochemical gradient and selectively inhibits nicotinamide adenine disphosphate: ubiquinone oxidoreductionase (complex I), thereby interfering with electron transport and

eventually generating oxygen radicals.

It has been demonstrated in tissue culture paradigms that FGF-2 (basic FGF) has trophic activity towards nigral dopaminergic neurons (Ferrari et al., Dev. Biol. 1989). Recently, Dr. Unsicker's group has demonstrated that administering FGF-2 in gel foam implants in the striatum results in the near complete protection of nigral dopaminergic neurons from the toxicity associated with MPTP exposure (Otto and Unsicker, J. Neuroscience, 1990).

Based on the data with FGF-2, an albumin fusion protein of the invention can be evaluated to determine whether it has an action similar to that of FGF-2 in enhancing dopaminergic neuronal survival *in vitro* and it can also be tested *in vivo* for protection of dopaminergic neurons in the striatum from the damage associated with MPTP treatment. The potential effect of an albumin fusion protein of the invention is first examined *in vitro* in a dopaminergic neuronal cell culture paradigm. The cultures are prepared by dissecting the midbrain floor plate from gestation day 14 Wistar rat embryos. The tissue is dissociated with trypsin and seeded at a density of 200,000 cells/cm<sup>2</sup> on polyorthinine-laminin coated glass coverslips. The cells are maintained in Dulbecco's Modified Eagle's medium and F12 medium containing hormonal supplements (N1). The cultures are fixed with paraformaldehyde after 8 days *in vitro* and are processed for tyrosine hydroxylase, a specific marker for dopaminergic neurons, immunohistochemical staining. Dissociated cell cultures are prepared from embryonic rats. The culture medium is changed every third day and the factors are also added at that time.

Since the dopaminergic neurons are isolated from animals at gestation day 14, a developmental time which is past the stage when the dopaminergic precursor cells are proliferating, an increase in the number of tyrosine hydroxylase immunopositive neurons would represent an increase in the number of dopaminergic neurons surviving *in vitro*. Therefore, if a therapeutic protein of the invention acts to prolong the survival of dopaminergic neurons, it would suggest that the fusion protein may be involved in Parkinson's Disease.

The studies described in this example tested activity of albumin fusion proteins of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of fusion proteins and polynucleotides of the invention (e.g., gene therapy).

**Example 24: The Effect of Albumin Fusion Proteins of the Invention on the Growth of Vascular Endothelial Cells**

On day 1, human umbilical vein endothelial cells (HUVEC) are seeded at  $2-5 \times 10^4$  cells/35 mm dish density in M199 medium containing 4% fetal bovine serum (FBS), 16 units/ml heparin, and 50 units/ml endothelial cell growth supplements (ECGS, Biotechnology, Inc.). On day 2, the medium is replaced with M199 containing 10% FBS, 8 units/ml heparin. An albumin fusion protein of the invention, and positive controls, such as VEGF and basic FGF (bFGF) are added, at varying concentrations. On days 4 and 6, the medium is replaced. On day 8, cell number is determined with a Coulter Counter.

An increase in the number of HUVEC cells indicates that the fusion protein may proliferate vascular endothelial cells, while a decrease in the number of HUVEC cells indicates that the fusion protein inhibits vascular endothelial cells.

The studies described in this example tested activity of an albumin fusion protein of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of a fusion protein and polynucleotides of the invention.

**Example 25: Rat Corneal Wound Healing Model**

This animal model shows the effect of an albumin fusion protein of the invention on neovascularization. The experimental protocol includes:

Making a 1-1.5 mm long incision from the center of cornea into the stromal layer.

Inserting a spatula below the lip of the incision facing the outer corner of the eye.

Making a pocket (its base is 1-1.5 mm from the edge of the eye).

Positioning a pellet, containing 50ng- 5ug of an albumin fusion protein of the invention, within the pocket.

Treatment with an albumin fusion protein of the invention can also be applied topically to the corneal wounds in a dosage range of 20mg - 500mg (daily treatment for five days).

The studies described in this example test the activity of an albumin fusion protein of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of fusion proteins and polynucleotides of the invention (e.g., gene therapy).

## Example 26: Diabetic Mouse and Glucocorticoid-Impaired Wound Healing Models

### *Diabetic db+/db+ Mouse Model.*

5 To demonstrate that an albumin fusion protein of the invention accelerates the healing process, the genetically diabetic mouse model of wound healing is used. The full thickness wound healing model in the db+/db+ mouse is a well characterized, clinically relevant and reproducible model of impaired wound healing. Healing of the diabetic wound is dependent on formation of granulation tissue and re-epithelialization rather than contraction (Gartner, M.H. *et al.*, *J. Surg. Res.* 52:389 (1992); Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)).

The diabetic animals have many of the characteristic features observed in Type II diabetes mellitus. Homozygous (db+/db+) mice are obese in comparison to their normal heterozygous (db+/+m) littermates. Mutant diabetic (db+/db+) mice have a single  
15 autosomal recessive mutation on chromosome 4 (db+) (Coleman *et al.* *Proc. Natl. Acad. Sci. USA* 77:283-293 (1982)). Animals show polyphagia, polydipsia and polyuria. Mutant diabetic mice (db+/db+) have elevated blood glucose, increased or normal insulin levels, and suppressed cell-mediated immunity (Mandel *et al.*, *J. Immunol.* 120:1375 (1978); Debray-Sachs, M. *et al.*, *Clin. Exp. Immunol.* 51(1):1-7 (1983); Leiter *et al.*, *Am. J. of*  
20 *Pathol.* 114:46-55 (1985)). Peripheral neuropathy, myocardial complications, and microvascular lesions, basement membrane thickening and glomerular filtration abnormalities have been described in these animals (Norido, F. *et al.*, *Exp. Neurol.* 83(2):221-232 (1984); Robertson *et al.*, *Diabetes* 29(1):60-67 (1980); Giacomelli *et al.*, *Lab Invest.* 40(4):460-473 (1979); Coleman, D.L., *Diabetes* 31 (Suppl):1-6 (1982)). These  
25 homozygous diabetic mice develop hyperglycemia that is resistant to insulin analogous to human type II diabetes (Mandel *et al.*, *J. Immunol.* 120:1375-1377 (1978)).

The characteristics observed in these animals suggests that healing in this model may be similar to the healing observed in human diabetes (Greenhalgh, *et al.*, *Am. J. of Pathol.* 136:1235-1246 (1990)).

30 Genetically diabetic female C57BL/KsJ (db+/db+) mice and their non-diabetic (db+/+m) heterozygous littermates are used in this study (Jackson Laboratories). The animals are purchased at 6 weeks of age and are 8 weeks old at the beginning of the study.

Animals are individually housed and received food and water ad libitum. All manipulations are performed using aseptic techniques. The experiments are conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory  
5 Animals.

Wounding protocol is performed according to previously reported methods (Tsuboi, R. and Rifkin, D.B., *J. Exp. Med.* 172:245-251 (1990)). Briefly, on the day of wounding, animals are anesthetized with an intraperitoneal injection of Avertin (0.01 mg/mL), 2,2,2-tribromoethanol and 2-methyl-2-butanol dissolved in deionized water. The  
10 dorsal region of the animal is shaved and the skin washed with 70% ethanol solution and iodine. The surgical area is dried with sterile gauze prior to wounding. An 8 mm full-thickness wound is then created using a Keyes tissue punch. Immediately following wounding, the surrounding skin is gently stretched to eliminate wound expansion. The wounds are left open for the duration of the experiment. Application of the treatment is  
15 given topically for 5 consecutive days commencing on the day of wounding. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of surgery and at two day intervals thereafter. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically  
20 using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

An albumin fusion protein of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

25 Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology and immunohistochemistry. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 diabetic and 5 non-diabetic controls) are  
30 evaluated: 1) Vehicle placebo control, 2) untreated group, and 3) treated group.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total square area of the wound. Contraction is then estimated by



establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is  $64\text{mm}^2$ , the corresponding size of the dermal punch. Calculations are made using the following formula:

5 [Open area on day 8] - [Open area on day 1] / [Open area on day 1]

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using a Reichert-Jung microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds are used to assess whether the healing process and the morphologic appearance of the repaired skin is altered by treatment with an albumin fusion protein of the invention. This assessment included verification of the presence of cell accumulation, inflammatory cells, capillaries, fibroblasts, re-epithelialization and epidermal maturity (Greenhalgh, D.G. *et al.*, *Am. J. Pathol.* 136:1235 (1990)). A calibrated lens micrometer is used by a blinded observer.

Tissue sections are also stained immunohistochemically with a polyclonal rabbit anti-human keratin antibody using ABC Elite detection system. Human skin is used as a positive tissue control while non-immune IgG is used as a negative control. Keratinocyte growth is determined by evaluating the extent of reepithelialization of the wound using a calibrated lens micrometer.

Proliferating cell nuclear antigen/cyclin (PCNA) in skin specimens is demonstrated by using anti-PCNA antibody (1:50) with an ABC Elite detection system. Human colon cancer served as a positive tissue control and human brain tissue is used as a negative tissue control. Each specimen included a section with omission of the primary antibody and substitution with non-immune mouse IgG. Ranking of these sections is based on the extent of proliferation on a scale of 0-8, the lower side of the scale reflecting slight proliferation to the higher side reflecting intense proliferation.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

### Steroid Impaired Rat Model

The inhibition of wound healing by steroids has been well documented in various

in vitro and in vivo systems (Wahl, Glucocorticoids and Wound healing. In: Anti-Inflammatory Steroid Action: Basic and Clinical Aspects. 280-302 (1989); Wahlet *et al.*, *J. Immunol.* 115: 476-481 (1975); Werb *et al.*, *J. Exp. Med.* 147:1684-1694 (1978)). Glucocorticoids retard wound healing by inhibiting angiogenesis, decreasing vascular permeability (Ebert *et al.*, *Am. Intern. Med.* 37:701-705 (1952)), fibroblast proliferation, and collagen synthesis (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978)) and producing a transient reduction of circulating monocytes (Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989)). The systemic administration of steroids to impaired wound healing is a well establish phenomenon in rats (Beck *et al.*, *Growth Factors.* 5: 295-304 (1991); Haynes *et al.*, *J. Clin. Invest.* 61: 703-797 (1978); Wahl, "Glucocorticoids and wound healing", In: Antiinflammatory Steroid Action: Basic and Clinical Aspects, Academic Press, New York, pp. 280-302 (1989); Pierce *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 2229-2233 (1989)).

To demonstrate that an albumin fusion protein of the invention can accelerate the healing process, the effects of multiple topical applications of the fusion protein on full thickness excisional skin wounds in rats in which healing has been impaired by the systemic administration of methylprednisolone is assessed.

Young adult male Sprague Dawley rats weighing 250-300 g (Charles River Laboratories) are used in this example. The animals are purchased at 8 weeks of age and are 9 weeks old at the beginning of the study. The healing response of rats is impaired by the systemic administration of methylprednisolone (17mg/kg/rat intramuscularly) at the time of wounding. Animals are individually housed and received food and water *ad libitum*. All manipulations are performed using aseptic techniques. This study is conducted according to the rules and guidelines of Human Genome Sciences, Inc. Institutional Animal Care and Use Committee and the Guidelines for the Care and Use of Laboratory Animals.

The wounding protocol is followed according to that described above. On the day of wounding, animals are anesthetized with an intramuscular injection of ketamine (50 mg/kg) and xylazine (5 mg/kg). The dorsal region of the animal is shaved and the skin washed with 70% ethanol and iodine solutions. The surgical area is dried with sterile

gauze prior to wounding. An 8 mm full-thickness wound is created using a Keyes tissue punch. The wounds are left open for the duration of the experiment. Applications of the testing materials are given topically once a day for 7 consecutive days commencing on the day of wounding and subsequent to methylprednisolone administration. Prior to treatment, wounds are gently cleansed with sterile saline and gauze sponges.

Wounds are visually examined and photographed at a fixed distance at the day of wounding and at the end of treatment. Wound closure is determined by daily measurement on days 1-5 and on day 8. Wounds are measured horizontally and vertically using a calibrated Jameson caliper. Wounds are considered healed if granulation tissue is no longer visible and the wound is covered by a continuous epithelium.

The fusion protein of the invention is administered using at a range different doses, from 4mg to 500mg per wound per day for 8 days in vehicle. Vehicle control groups received 50mL of vehicle solution.

Animals are euthanized on day 8 with an intraperitoneal injection of sodium pentobarbital (300mg/kg). The wounds and surrounding skin are then harvested for histology. Tissue specimens are placed in 10% neutral buffered formalin in tissue cassettes between biopsy sponges for further processing.

Three groups of 10 animals each (5 with methylprednisolone and 5 without glucocorticoid) are evaluated: 1) Untreated group 2) Vehicle placebo control 3) treated groups.

Wound closure is analyzed by measuring the area in the vertical and horizontal axis and obtaining the total area of the wound. Closure is then estimated by establishing the differences between the initial wound area (day 0) and that of post treatment (day 8). The wound area on day 1 is 64mm<sup>2</sup>, the corresponding size of the dermal punch. Calculations are made using the following formula:

$$[\text{Open area on day 8}] - [\text{Open area on day 1}] / [\text{Open area on day 1}]$$

Specimens are fixed in 10% buffered formalin and paraffin embedded blocks are sectioned perpendicular to the wound surface (5mm) and cut using an Olympus microtome. Routine hematoxylin-eosin (H&E) staining is performed on cross-sections of bisected wounds. Histologic examination of the wounds allows assessment of whether the

healing process and the morphologic appearance of the repaired skin is improved by treatment with an albumin fusion protein of the invention. A calibrated lens micrometer is used by a blinded observer to determine the distance of the wound gap.

Experimental data are analyzed using an unpaired t test. A p value of  $< 0.05$  is considered significant.

The studies described in this example tested activity of an albumin fusion protein of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of fusion proteins and polynucleotides of the invention (e.g., gene therapy).

#### **Example 27: Lymphedema Animal Model**

The purpose of this experimental approach is to create an appropriate and consistent lymphedema model for testing the therapeutic effects of an albumin fusion protein of the invention in lymphangiogenesis and re-establishment of the lymphatic circulatory system in the rat hind limb. Effectiveness is measured by swelling volume of the affected limb, quantification of the amount of lymphatic vasculature, total blood plasma protein, and histopathology. Acute lymphedema is observed for 7-10 days. Perhaps more importantly, the chronic progress of the edema is followed for up to 3-4 weeks.

Prior to beginning surgery, blood sample is drawn for protein concentration analysis. Male rats weighing approximately ~350g are dosed with Pentobarbital. Subsequently, the right legs are shaved from knee to hip. The shaved area is swabbed with gauze soaked in 70% EtOH. Blood is drawn for serum total protein testing. Circumference and volumetric measurements are made prior to injecting dye into paws after marking 2 measurement levels (0.5 cm above heel, at mid-pt of dorsal paw). The intradermal dorsum of both right and left paws are injected with 0.05 ml of 1% Evan's Blue. Circumference and volumetric measurements are then made following injection of dye into paws.

Using the knee joint as a landmark, a mid-leg inguinal incision is made circumferentially allowing the femoral vessels to be located. Forceps and hemostats are used to dissect and separate the skin flaps. After locating the femoral vessels, the lymphatic vessel that runs along side and underneath the vessel(s) is located. The main

lymphatic vessels in this area are then electrically coagulated or suture ligated.

Using a microscope, muscles in back of the leg (near the semitendinosus and adductors) are bluntly dissected. The popliteal lymph node is then located. The 2 proximal and 2 distal lymphatic vessels and distal blood supply of the popliteal node are then ligated by suturing. The popliteal lymph node, and any accompanying adipose tissue, is then removed by cutting connective tissues.

Care is taken to control any mild bleeding resulting from this procedure. After lymphatics are occluded, the skin flaps are sealed by using liquid skin (Vetbond) (AJ Buck). The separated skin edges are sealed to the underlying muscle tissue while leaving a gap of ~0.5 cm around the leg. Skin also may be anchored by suturing to underlying muscle when necessary.

To avoid infection, animals are housed individually with mesh (no bedding). Recovering animals are checked daily through the optimal edematous peak, which typically occurred by day 5-7. The plateau edematous peak are then observed. To evaluate the intensity of the lymphedema, the circumference and volumes of 2 designated places on each paw before operation and daily for 7 days are measured. The effect of plasma proteins on lymphedema is determined and whether protein analysis is a useful testing perimeter is also investigated. The weights of both control and edematous limbs are evaluated at 2 places. Analysis is performed in a blind manner.

**Circumference Measurements:** Under brief gas anesthetic to prevent limb movement, a cloth tape is used to measure limb circumference. Measurements are done at the ankle bone and dorsal paw by 2 different people and those 2 readings are averaged. Readings are taken from both control and edematous limbs.

**Volumetric Measurements:** On the day of surgery, animals are anesthetized with Pentobarbital and are tested prior to surgery. For daily volumetrics animals are under brief halothane anesthetic (rapid immobilization and quick recovery), and both legs are shaved and equally marked using waterproof marker on legs. Legs are first dipped in water, then dipped into instrument to each marked level then measured by Buxco edema software(Chen/Victor). Data is recorded by one person, while the other is dipping the limb to marked area.

**Blood-plasma protein measurements:** Blood is drawn, spun, and serum separated prior to surgery and then at conclusion for total protein and  $\text{Ca}^{2+}$  comparison.

Limb Weight Comparison: After drawing blood, the animal is prepared for tissue collection. The limbs are amputated using a quilltine, then both experimental and control legs are cut at the ligature and weighed. A second weighing is done as the tibio-cacaneal joint is disarticulated and the foot is weighed.

5        Histological Preparations: The transverse muscle located behind the knee (popliteal) area is dissected and arranged in a metal mold, filled with freezeGel, dipped into cold methylbutane, placed into labeled sample bags at - 80EC until sectioning. Upon sectioning, the muscle is observed under fluorescent microscopy for lymphatics..

10        The studies described in this example tested activity of fusion proteins of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of fusion protein and polynucleotides of the invention (e.g., gene therapy).

**Example 28: Suppression of TNF alpha-Induced Adhesion Molecule Expression by an Albumin Fusion Protein of the Invention**

15        The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules (CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and  
20        endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the  
25        expression of these CAMs.

Tumor necrosis factor alpha (TNF-a), a potent proinflammatory cytokine, is a stimulator of all three CAMs on endothelial cells and may be involved in a wide variety of inflammatory responses, often resulting in a pathological outcome.

30        The potential of an albumin fusion protein of the invention to mediate a suppression of TNF-a induced CAM expression can be examined. A modified ELISA assay which uses ECs as a solid phase absorbent is employed to measure the amount of CAM expression on TNF-a treated ECs when co-stimulated with a member of the FGF

family of proteins.

To perform the experiment, human umbilical vein endothelial cell (HUVEC) cultures are obtained from pooled cord harvests and maintained in growth medium (EGM-2; Clonetics, San Diego, CA) supplemented with 10% FCS and 1% penicillin/streptomycin in a 37 degree C humidified incubator containing 5% CO<sub>2</sub>. HUVECs are seeded in 96-well plates at concentrations of  $1 \times 10^4$  cells/well in EGM medium at 37 degree C for 18-24 hrs or until confluent. The monolayers are subsequently washed 3 times with a serum-free solution of RPMI-1640 supplemented with 100 U/ml penicillin and 100 mg/ml streptomycin, and treated with a given cytokine and/or growth factor(s) for 24 h at 37 degree C. Following incubation, the cells are then evaluated for CAM expression.

Human Umbilical Vein Endothelial cells (HUVECs) are grown in a standard 96 well plate to confluence. Growth medium is removed from the cells and replaced with 90 ul of 199 Medium (10% FBS). Samples for testing and positive or negative controls are added to the plate in triplicate (in 10 ul volumes). Plates are incubated at 37 degree C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100 ul of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C for 30 min.

Fixative is then removed from the wells and wells are washed 1X with PBS(+Ca,Mg)+0.5% BSA and drained. Do not allow the wells to dry. Add 10 ul of diluted primary antibody to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10 µg/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA.

Then add 20 µl of diluted ExtrAvidin-Alkaline Phosphotase (1:5,000 dilution) to each well and incubated at 37°C for 30 min. Wells are washed X3 with PBS(+Ca,Mg)+0.5% BSA. 1 tablet of p-Nitrophenol Phosphate pNPP is dissolved in 5 ml of glycine buffer (pH 10.4). 100 µl of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphotase in glycine buffer:  $1:5,000 (10^0) > 10^{-0.5} > 10^{-1} > 10^{-1.5}$ . 5 µl of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100 µl of pNPP reagent must then be added to each of

the standard wells. The plate must be incubated at 37°C for 4h. A volume of 50 µl of 3M NaOH is added to all wells. The results are quantified on a plate reader at 405 nm. The background subtraction option is used on blank wells filled with glycine buffer only. The template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

The studies described in this example tested activity of fusion proteins of the invention. However, one skilled in the art could easily modify the exemplified studies to test the activity of fusion proteins and polynucleotides of the invention (e.g., gene therapy).

### Example 29: Construction of GAS Reporter Construct

One signal transduction pathway involved in the differentiation and proliferation of cells is called the Jaks-STATs pathway. Activated proteins in the Jaks-STATs pathway bind to gamma activation site "GAS" elements or interferon-sensitive responsive element ("ISRE"), located in the promoter of many genes. The binding of a protein to these elements alter the expression of the associated gene.

GAS and ISRE elements are recognized by a class of transcription factors called Signal Transducers and Activators of Transcription, or "STATs." There are six members of the STATs family. Stat1 and Stat3 are present in many cell types, as is Stat2 (as response to IFN-alpha is widespread). Stat4 is more restricted and is not in many cell types though it has been found in T helper class I, cells after treatment with IL-12. Stat5 was originally called mammary growth factor, but has been found at higher concentrations in other cells including myeloid cells. It can be activated in tissue culture cells by many cytokines.

The STATs are activated to translocate from the cytoplasm to the nucleus upon tyrosine phosphorylation by a set of kinases known as the Janus Kinase ("Jaks") family. Jaks represent a distinct family of soluble tyrosine kinases and include Tyk2, Jak1, Jak2, and Jak3. These kinases display significant sequence similarity and are generally catalytically inactive in resting cells.

The Jaks are activated by a wide range of receptors summarized in the Table below. (Adapted from review by Schidler and Darnell, Ann. Rev. Biochem. 64:621-51



(1995)). A cytokine receptor family, capable of activating Jaks, is divided into two groups: (a) Class 1 includes receptors for IL-2, IL-3, IL-4, IL-6, IL-7, IL-9, IL-11, IL-12, IL-15, Epo, PRL, GH, G-CSF, GM-CSF, LIF, CNTF, and thrombopoietin; and (b) Class 2 includes IFN-a, IFN-g, and IL-10. The Class 1 receptors share a conserved cysteine motif (a set of four conserved cysteines and one tryptophan) and a WSXWS motif (a membrane proximal region encoding Trp-Ser-Xaa-Trp-Ser (SEQ ID NO: 37)).

Thus, on binding of a ligand to a receptor, Jaks are activated, which in turn activate STATs, which then translocate and bind to GAS elements. This entire process is encompassed in the Jaks-STATs signal transduction pathway. Therefore, activation of the Jaks-STATs pathway, reflected by the binding of the GAS or the ISRE element, can be used to indicate proteins involved in the proliferation and differentiation of cells. For example, growth factors and cytokines are known to activate the Jaks-STATs pathway (See Table below). Thus, by using GAS elements linked to reporter molecules, activators of the Jaks-STATs pathway can be identified.

<u>Ligand</u>	<u>JAKs</u>		<u>STATs</u>			<u>GAS(elements) or ISRE</u>
	<u>tyk2</u>	<u>Jak1</u>	<u>Jak2</u>	<u>Jak3</u>		
<u>IFN family</u>						
IFN-a/B	+	+	-	-	1,2,3	ISRE
IFN-g		+	+	-	1	GAS (IRF1>Lys6>IFP)
Il-10	+	?	?	-	1,3	
<u>gp130 family</u>						
IL-6 (Pleiotropic)	+	+	+	?	1,3	GAS (IRF1>Lys6>IFP)
Il-11(Pleiotropic)	?	+	?	?	1,3	
OnM(Pleiotropic)	?	+	+	?	1,3	
LIF(Pleiotropic)	?	+	+	?	1,3	
CNTF(Pleiotropic)	-/+	+	+	?	1,3	
G-CSF(Pleiotropic)	?	+	?	?	1,3	
IL-12(Pleiotropic)	+	-	+	+	1,3	
<u>g-C family</u>						
IL-2 (lymphocytes)	-	+	-	+	1,3,5	GAS
IL-4 (lymph/myeloid) >>Ly6(IgH)	-	+	-	+	6	GAS (IRF1 = IFP)
IL-7 (lymphocytes)	-	+	-	+	5	GAS
IL-9 (lymphocytes)	-	+	-	+	5	GAS
IL-13 (lymphocyte)	-	+	?	?	6	GAS
IL-15	?	+	?	+	5	GAS
<u>gp140 family</u>						
IL-3 (myeloid) (IRF1>IFP>>Ly6)	-	-	+	-	5	GAS
IL-5 (myeloid)	-	-	+	-	5	GAS
GM-CSF (myeloid)	-	-	+	-	5	GAS
<u>Growth hormone family</u>						
GH	?	-	+	-	5	
PRL	?	+/-	+	-	1,3,5	
EPO	?	-	+	-	5	GAS(B-IFP)
CAS>IRF1=IFP>>Ly6)						
<u>Receptor Tyrosine Kinases</u>						
EGF	?	+	+	-	1,3	GAS (IRF1)
PDGF	?	+	+	-	1,3	
CSF-1	?	+	+	-	1,3	GAS (not IRF1)

To construct a synthetic GAS containing promoter element, which is used in the Biological Assays described in Examples 32-33, a PCR based strategy is employed to generate a GAS-SV40 promoter sequence. The 5' primer contains four tandem copies of the GAS binding site found in the IRF1 promoter and previously demonstrated to bind 5 STATs upon induction with a range of cytokines (Rothman et al., Immunity 1:457-468 (1994).), although other GAS or ISRE elements can be used instead. The 5' primer also contains 18bp of sequence complementary to the SV40 early promoter sequence and is flanked with an XhoI site. The sequence of the 5' primer is:

5':GCGCCTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTC  
10 CCGAAATGATTTCCTCCGAAATATCTGCCATCTCAATTAG:3' (SEQ ID NO: 38)

The downstream primer is complementary to the SV40 promoter and is flanked with a Hind III site: 5':GCGGCAAGCTTTTGTCAAAGCCTAGGC:3' (SEQ ID NO: 39)

PCR amplification is performed using the SV40 promoter template present in the B-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested 15 with XhoI/Hind III and subcloned into BLSK2-. (Stratagene.) Sequencing with forward and reverse primers confirms that the insert contains the following sequence:

5':CTCGAGATTTCCTCCGAAATCTAGATTTCCTCCGAAATGATTTCCTCCGA  
AATGATTTCCTCCGAAATATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCG  
CCCCTAACTCCGCCCATCCCGCCCCTAACTCCGCCCAGTTCGCCCATTCTCCG  
20 CCCCATGGCTGACTAATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCC  
TCTGAGCTATTCCAGAAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTG  
CAAAAAGCTT:3' (SEQ ID NO: 40)

With this GAS promoter element linked to the SV40 promoter, a GAS:SEAP2 reporter construct is next engineered. Here, the reporter molecule is a secreted alkaline 25 phosphatase, or "SEAP." Clearly, however, any reporter molecule can be instead of SEAP, in this or in any of the other Examples. Well known reporter molecules that can be used instead of SEAP include chloramphenicol acetyltransferase (CAT), luciferase, alkaline phosphatase, B-galactosidase, green fluorescent protein (GFP), or any protein detectable by an antibody.

30 The above sequence confirmed synthetic GAS-SV40 promoter element is subcloned into the pSEAP-Promoter vector obtained from Clontech using HindIII and XhoI, effectively replacing the SV40 promoter with the amplified GAS:SV40 promoter

element, to create the GAS-SEAP vector. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

Thus, in order to generate mammalian stable cell lines expressing the GAS-SEAP reporter, the GAS-SEAP cassette is removed from the GAS-SEAP vector using SalI and NotI, and inserted into a backbone vector containing the neomycin resistance gene, such as pGFP-1 (Clontech), using these restriction sites in the multiple cloning site, to create the GAS-SEAP/Neo vector. Once this vector is transfected into mammalian cells, this vector can then be used as a reporter molecule for GAS binding as described in Examples 32-33.

Other constructs can be made using the above description and replacing GAS with a different promoter sequence. For example, construction of reporter molecules containing EGR and NF-KB promoter sequences are described in Examples 34 and 35. However, many other promoters can be substituted using the protocols described in these Examples. For instance, SRE, IL-2, NFAT, or Osteocalcin promoters can be substituted, alone or in combination (e.g., GAS/NF-KB/EGR, GAS/NF-KB, IL-2/NFAT, or NF-KB/GAS). Similarly, other cell lines can be used to test reporter construct activity, such as HELA (epithelial), HUVEC (endothelial), Reh (B-cell), Saos-2 (osteoblast), HUVAC (aortic), or Cardiomyocyte.

#### **Example 30: Assay for SEAP Activity**

As a reporter molecule for the assays described in examples disclosed herein, SEAP activity is assayed using the Tropix Phospho-light Kit (Cat. BP-400) according to the following general procedure. The Tropix Phospho-light Kit supplies the Dilution, Assay, and Reaction Buffers used below.

Prime a dispenser with the 2.5x Dilution Buffer and dispense 15 ul of 2.5x dilution buffer into Optiplates containing 35 ul of a solution containing an albumin fusion protein of the invention. Seal the plates with a plastic sealer and incubate at 65 degree C for 30 min. Separate the Optiplates to avoid uneven heating.

Cool the samples to room temperature for 15 minutes. Empty the dispenser and prime with the Assay Buffer. Add 50 ml Assay Buffer and incubate at room temperature 5 min. Empty the dispenser and prime with the Reaction Buffer (see the Table below). Add 50 ul Reaction Buffer and incubate at room temperature for 20 minutes. Since the

intensity of the chemiluminescent signal is time dependent, and it takes about 10 minutes to read 5 plates on a luminometer, thus one should treat 5 plates at each time and start the second set 10 minutes later.

- 5 Read the relative light unit in the luminometer. Set H12 as blank, and print the results. An increase in chemiluminescence indicates reporter activity.

Reaction Buffer Formulation:

# plates	of (ml)	Rxn buffer diluent (ml)	CSPD
10	60		3
11	65		3.25
12	70		3.5
13	75		3.75
14	80		4
15	85		4.25
16	90		4.5
17	95		4.75
18	100		5
19	105		5.25
20	110		5.5
21	115		5.75
22	120		6
23	125		6.25
24	130		6.5
25	135		6.75
26	140		7
27	145		7.25
28	150		7.5
29	155		7.75
30	160		8
31	165		8.25
32	170		8.5

33	175	8.75
34	180	9
35	185	9.25
36	190	9.5
37	195	9.75
38	200	10
39	205	10.25
40	210	10.5
41	215	10.75
42	220	11
43	225	11.25
44	230	11.5
45	235	11.75
46	240	12
47	245	12.25
48	250	12.5
49	255	12.75
50	260	13

### Example 31: Assay Identifying Neuronal Activity.

When cells undergo differentiation and proliferation, a group of genes are activated through many different signal transduction pathways. One of these genes, EGR1 (early growth response gene 1), is induced in various tissues and cell types upon activation. The promoter of EGR1 is responsible for such induction. Using the EGR1 promoter linked to reporter molecules, the ability of fusion proteins of the invention to activate cells can be assessed.

Particularly, the following protocol is used to assess neuronal activity in PC12 cell lines. PC12 cells (rat pheochromocytoma cells) are known to proliferate and/or differentiate by activation with a number of mitogens, such as TPA (tetradecanoyl phorbol acetate), NGF (nerve growth factor), and EGF (epidermal growth factor). The EGR1 gene expression is activated during this treatment. Thus, by stably transfecting PC12 cells with

a construct containing an EGR promoter linked to SEAP reporter, activation of PC12 cells by an albumin fusion protein of the present invention can be assessed.

The EGR/SEAP reporter construct can be assembled by the following protocol. The EGR-1 promoter sequence (-633 to +1)(Sakamoto K et al., Oncogene 6:867-871 (1991)) can be PCR amplified from human genomic DNA using the following primers:

5' GCGCTCGAGGGATGACAGCGATAGAACCCCGG-3' (SEQ ID NO: 41)

5' GCGAAGCTTCGCGACTCCCCGGATCCGCCTC-3' (SEQ ID NO: 42)

Using the GAS:SEAP/Neo vector produced in Example 29, EGR1 amplified product can then be inserted into this vector. Linearize the GAS:SEAP/Neo vector using restriction enzymes XhoI/HindIII, removing the GAS/SV40 stuffer. Restrict the EGR1 amplified product with these same enzymes. Ligate the vector and the EGR1 promoter.

To prepare 96 well-plates for cell culture, two mls of a coating solution (1:30 dilution of collagen type I (Upstate Biotech Inc. Cat#08-115) in 30% ethanol (filter sterilized)) is added per one 10 cm plate or 50 ml per well of the 96-well plate, and allowed to air dry for 2 hr.

PC12 cells are routinely grown in RPMI-1640 medium (Bio Whittaker) containing 10% horse serum (JRH BIOSCIENCES, Cat. # 12449-78P), 5% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 ug/ml streptomycin on a precoated 10 cm tissue culture dish. One to four split is done every three to four days. Cells are removed from the plates by scraping and resuspended with pipetting up and down for more than 15 times.

Transfect the EGR/SEAP/Neo construct into PC12 using techniques known in the art. EGR-SEAP/PC12 stable cells are obtained by growing the cells in 300 ug/ml G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 300 ug/ml G418 for couple of passages.

To assay for neuronal activity, a 10 cm plate with cells around 70 to 80% confluent is screened by removing the old medium. Wash the cells once with PBS (Phosphate buffered saline). Then starve the cells in low serum medium (RPMI-1640 containing 1% horse serum and 0.5% FBS with antibiotics) overnight.

The next morning, remove the medium and wash the cells with PBS. Scrape off the cells from the plate, suspend the cells well in 2 ml low serum medium. Count the cell number and add more low serum medium to reach final cell density as  $5 \times 10^5$  cells/ml.

09833249-041241  
"02T40" sheet 960

Add 200 ul of the cell suspension to each well of 96-well plate (equivalent to  $1 \times 10^5$  cells/well). Add a series of different concentrations of an albumin fusion protein of the invention, 37 degree C for 48 to 72 hr. As a positive control, a growth factor known to activate PC12 cells through EGR can be used, such as 50 ng/ul of Neuronal Growth Factor (NGF). Over fifty-fold induction of SEAP is typically seen in the positive control wells. SEAP assay may be routinely performed using techniques known in the art and/or as described in Example 30.

#### Example 32: Assay for T-cell Activity.

10 The following protocol is used to assess T-cell activity by identifying factors, and determining whether an albumin fusion protein of the invention proliferates and/or differentiates T-cells. T-cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 29. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The T-cell used in this assay is  
15 Jurkat T-cells (ATCC Accession No. TIB-152), although Molt-3 cells (ATCC Accession No. CRL-1552) and Molt-4 cells (ATCC Accession No. CRL-1582) cells can also be used.

Jurkat T-cells are lymphoblastic CD4+ Th1 helper cells. In order to generate stable cell lines, approximately 2 million Jurkat cells are transfected with the GAS-SEAP/neo vector using DMRIE-C (Life Technologies)(transfection procedure described below).  
20 The transfected cells are seeded to a density of approximately 20,000 cells per well and transfectants resistant to 1 mg/ml gentamicin selected. Resistant colonies are expanded and then tested for their response to increasing concentrations of interferon gamma. The dose response of a selected clone is demonstrated.

Specifically, the following protocol will yield sufficient cells for 75 wells  
25 containing 200 ul of cells. Thus, it is either scaled up, or performed in multiple to generate sufficient cells for multiple 96 well plates. Jurkat cells are maintained in RPMI + 10% serum with 1%Pen-Strep. Combine 2.5 mls of OPTI-MEM (Life Technologies) with 10 ug of plasmid DNA in a T25 flask. Add 2.5 ml OPTI-MEM containing 50 ul of DMRIE-C and incubate at room temperature for 15-45 mins.

30 During the incubation period, count cell concentration, spin down the required number of cells ( $10^7$  per transfection), and resuspend in OPTI-MEM to a final concentration of  $10^7$  cells/ml. Then add 1ml of  $1 \times 10^7$  cells in OPTI-MEM to T25 flask



and incubate at 37 degree C for 6 hrs. After the incubation, add 10 ml of RPMI + 15% serum.

The Jurkat:GAS-SEAP stable reporter lines are maintained in RPMI + 10% serum, 1 mg/ml Genticin, and 1% Pen-Strep. These cells are treated with varying concentrations of one or more fusion proteins of the present invention.

On the day of treatment with the fusion protein, the cells should be washed and resuspended in fresh RPMI + 10% serum to a density of 500,000 cells per ml. The exact number of cells required will depend on the number of fusion proteins and the number of different concentrations of fusion proteins being screened. For one 96 well plate, approximately 10 million cells (for 10 plates, 100 million cells) are required.

The well dishes containing Jurkat cells treated with the fusion protein are placed in an incubator for 48 hrs (note: this time is variable between 48-72 hrs). 35 ul samples from each well are then transferred to an opaque 96 well plate using a 12 channel pipette. The opaque plates should be covered (using sellophene covers) and stored at -20 degree C until SEAP assays are performed according to Example 30. The plates containing the remaining treated cells are placed at 4 degree C and serve as a source of material for repeating the assay on a specific well if desired.

As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate Jurkat T cells. Over 30 fold induction is typically observed in the positive control wells.

The above protocol may be used in the generation of both transient, as well as, stable transfected cells, which would be apparent to those of skill in the art.

### **Example 33: Assay for T-cell Activity**

NF-KB (Nuclear Factor KB) is a transcription factor activated by a wide variety of agents including the inflammatory cytokines IL-1 and TNF, CD30 and CD40, lymphotoxin-alpha and lymphotoxin-beta, by exposure to LPS or thrombin, and by expression of certain viral gene products. As a transcription factor, NF-KB regulates the expression of genes involved in immune cell activation, control of apoptosis (NF- KB appears to shield cells from apoptosis), B and T-cell development, anti-viral and antimicrobial responses, and multiple stress responses.

In non-stimulated conditions, NF- KB is retained in the cytoplasm with I-KB.

(Inhibitor KB). However, upon stimulation, I- KB is phosphorylated and degraded, causing NF- KB to shuttle to the nucleus, thereby activating transcription of target genes. Target genes activated by NF- KB include IL-2, IL-6, GM-CSF, ICAM-1 and class 1 MHC.

5 Due to its central role and ability to respond to a range of stimuli, reporter constructs utilizing the NF-KB promoter element are used to screen the fusion protein. Activators or inhibitors of NF-KB would be useful in treating, preventing, and/or diagnosing diseases. For example, inhibitors of NF-KB could be used to treat those diseases related to the acute or chronic activation of NF-KB, such as rheumatoid arthritis.

10 To construct a vector containing the NF-KB promoter element, a PCR based strategy is employed. The upstream primer contains four tandem copies of the NF-KB binding site (GGGGACTTTCCC) (SEQ ID NO: 43), 18 bp of sequence complementary to the 5' end of the SV40 early promoter sequence, and is flanked with an XhoI site:

5':GCGGCCTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGG  
15 ACTTTCCATCCTGCCATCTCAATTAG:3' (SEQ ID NO: 44)

The downstream primer is complementary to the 3' end of the SV40 promoter and is flanked with a Hind III site:

5':GCGGCAAGCTTTTTGCAAAGCCTAGGC:3' (SEQ ID NO: 39)

PCR amplification is performed using the SV40 promoter template present in the  
20 pB-gal:promoter plasmid obtained from Clontech. The resulting PCR fragment is digested with XhoI and Hind III and subcloned into BLSK2-. (Stratagene) Sequencing with the T7 and T3 primers confirms the insert contains the following sequence:

5':CTCGAGGGGACTTTCCCGGGGACTTTCCGGGGACTTTCCGGGACTTT  
CCATCTGCCATCTCAATTAGTCAGCAACCATAGTCCCGCCCCTAACTCCGCCC  
25 ATCCCGCCCCTAACTCCGCCCAGTTCGCCCATTCTCCGCCCCATGGCTGACTA  
ATTTTTTTTATTTATGCAGAGGCCGAGGCCGCTCGGCCTCTGAGCTATTCCAG  
AAGTAGTGAGGAGGCTTTTTTGGAGGCCTAGGCTTTTGCAAAAAGCTT:3' (SEQ  
ID NO: 45)

Next, replace the SV40 minimal promoter element present in the pSEAP2-  
30 promoter plasmid (Clontech) with this NF-KB/SV40 fragment using XhoI and HindIII. However, this vector does not contain a neomycin resistance gene, and therefore, is not preferred for mammalian expression systems.

0903345-04301  
FOI b7D, b7E, b7C

In order to generate stable mammalian cell lines, the NF-KB/SV40/SEAP cassette is removed from the above NF-KB/SEAP vector using restriction enzymes Sall and NotI, and inserted into a vector containing neomycin resistance. Particularly, the NF-KB/SV40/SEAP cassette was inserted into pGFP-1 (Clontech), replacing the GFP gene, after restricting pGFP-1 with Sall and NotI.

Once NF-KB/SV40/SEAP/Neo vector is created, stable Jurkat T-cells are created and maintained according to the protocol described in Example 32. Similarly, the method for assaying fusion proteins with these stable Jurkat T-cells is also described in Example 32. As a positive control, exogenous TNF alpha (0.1, 1, 10 ng) is added to wells H9, H10, and H11, with a 5-10 fold activation typically observed.

### Example 33: Assay Identifying Myeloid Activity

The following protocol is used to assess myeloid activity of an albumin fusion protein of the present invention by determining whether the fusion protein proliferates and/or differentiates myeloid cells. Myeloid cell activity is assessed using the GAS/SEAP/Neo construct produced in Example 29. Thus, factors that increase SEAP activity indicate the ability to activate the Jaks-STATS signal transduction pathway. The myeloid cell used in this assay is U937, a pre-monocyte cell line, although TF-1, HL60, or KG1 can be used.

To transiently transfect U937 cells with the GAS/SEAP/Neo construct produced in Example 29, a DEAE-Dextran method (Kharbanda et. al., 1994, Cell Growth & Differentiation, 5:259-265) is used. First, harvest  $2 \times 10^7$  U937 cells and wash with PBS. The U937 cells are usually grown in RPMI 1640 medium containing 10% heat-inactivated fetal bovine serum (FBS) supplemented with 100 units/ml penicillin and 100 mg/ml streptomycin.

Next, suspend the cells in 1 ml of 20 mM Tris-HCl (pH 7.4) buffer containing 0.5 mg/ml DEAE-Dextran, 8 ug GAS-SEAP2 plasmid DNA, 140 mM NaCl, 5 mM KCl, 375 uM  $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ , 1 mM  $\text{MgCl}_2$ , and 675 uM  $\text{CaCl}_2$ . Incubate at 37 degrees C for 45 min.

Wash the cells with RPMI 1640 medium containing 10% FBS and then resuspend in 10 ml complete medium and incubate at 37 degree C for 36 hr.

The GAS-SEAP/U937 stable cells are obtained by growing the cells in 400 ug/ml

G418. The G418-free medium is used for routine growth but every one to two months, the cells should be re-grown in 400 ug/ml G418 for couple of passages.

These cells are tested by harvesting  $1 \times 10^8$  cells (this is enough for ten 96-well plates assay) and wash with PBS. Suspend the cells in 200 ml above described growth medium, with a final density of  $5 \times 10^5$  cells/ml. Plate 200 ul cells per well in the 96-well plate (or  $1 \times 10^5$  cells/well).

Add different concentrations of the fusion protein. Incubate at 37 degree C for 48 to 72 hr. As a positive control, 100 Unit/ml interferon gamma can be used which is known to activate U937 cells. Over 30 fold induction is typically observed in the positive control wells. SEAP assay the supernatant according to methods known in the art and/or the protocol described in Example 30.

#### **Example 34: Assay Identifying Changes in Small Molecule Concentration and Membrane Permeability**

Binding of a ligand to a receptor is known to alter intracellular levels of small molecules, such as calcium, potassium, sodium, and pH, as well as alter membrane potential. These alterations can be measured in an assay to identify fusion proteins which bind to receptors of a particular cell. Although the following protocol describes an assay for calcium, this protocol can easily be modified to detect changes in potassium, sodium, pH, membrane potential, or any other small molecule which is detectable by a fluorescent probe.

The following assay uses Fluorometric Imaging Plate Reader ("FLIPR") to measure changes in fluorescent molecules (Molecular Probes) that bind small molecules. Clearly, any fluorescent molecule detecting a small molecule can be used instead of the calcium fluorescent molecule, fluo-4 (Molecular Probes, Inc.; catalog no. F-14202), used here.

For adherent cells, seed the cells at 10,000 -20,000 cells/well in a Co-star black 96-well plate with clear bottom. The plate is incubated in a CO<sub>2</sub> incubator for 20 hours. The adherent cells are washed two times in Biotek washer with 200 ul of HBSS (Hank's Balanced Salt Solution) leaving 100 ul of buffer after the final wash.

A stock solution of 1 mg/ml fluo-4 is made in 10% pluronic acid DMSO. To load the cells with fluo-4, 50 ul of 12 ug/ml fluo-4 is added to each well. The plate is incubated

at 37 degrees C in a CO<sub>2</sub> incubator for 60 min. The plate is washed four times in the Biotek washer with HBSS leaving 100 ul of buffer.

For non-adherent cells, the cells are spun down from culture media. Cells are re-suspended to 2-5x10<sup>6</sup> cells/ml with HBSS in a 50-ml conical tube. 4 ul of 1 mg/ml fluo-4 solution in 10% pluronic acid DMSO is added to each ml of cell suspension. The tube is then placed in a 37 degrees C water bath for 30-60 min. The cells are washed twice with HBSS, resuspended to 1x10<sup>6</sup> cells/ml, and dispensed into a microplate, 100 ul/well. The plate is centrifuged at 1000 rpm for 5 min. The plate is then washed once in Denley Cell Wash with 200 ul, followed by an aspiration step to 100 ul final volume.

For a non-cell based assay, each well contains a fluorescent molecule, such as fluo-4. The fusion protein of the invention is added to the well, and a change in fluorescence is detected.

To measure the fluorescence of intracellular calcium, the FLIPR is set for the following parameters: (1) System gain is 300-800 mW; (2) Exposure time is 0.4 second; (3) Camera F/stop is F/2; (4) Excitation is 488 nm; (5) Emission is 530 nm; and (6) Sample addition is 50 ul. Increased emission at 530 nm indicates an extracellular signaling event caused by an albumin fusion protein of the present invention or a molecule induced by an albumin fusion protein of the present invention, which has resulted in an increase in the intracellular Ca<sup>++</sup> concentration.

#### **Example 35: Assay Identifying Tyrosine Kinase Activity**

The Protein Tyrosine Kinases (PTK) represent a diverse group of transmembrane and cytoplasmic kinases. Within the Receptor Protein Tyrosine Kinase (RPTK) group are receptors for a range of mitogenic and metabolic growth factors including the PDGF, FGF, EGF, NGF, HGF and Insulin receptor subfamilies. In addition there are a large family of RPTKs for which the corresponding ligand is unknown. Ligands for RPTKs include mainly secreted small proteins, but also membrane-bound and extracellular matrix proteins.

Activation of RPTK by ligands involves ligand-mediated receptor dimerization, resulting in transphosphorylation of the receptor subunits and activation of the cytoplasmic tyrosine kinases. The cytoplasmic tyrosine kinases include receptor associated tyrosine kinases of the src-family (e.g., src, yes, lck, lyn, fyn) and non-receptor linked and cytosolic

protein tyrosine kinases, such as the Jak family, members of which mediate signal transduction triggered by the cytokine superfamily of receptors (e.g., the Interleukins, Interferons, GM-CSF, and Leptin).

Because of the wide range of known factors capable of stimulating tyrosine kinase activity, identifying whether an albumin fusion protein of the present invention or a molecule induced by a fusion protein of the present invention is capable of activating tyrosine kinase signal transduction pathways is of interest. Therefore, the following protocol is designed to identify such molecules capable of activating the tyrosine kinase signal transduction pathways.

Seed target cells (e.g., primary keratinocytes) at a density of approximately 25,000 cells per well in a 96 well Loprodyne Silent Screen Plates purchased from Nalge Nunc (Naperville, IL). The plates are sterilized with two 30 minute rinses with 100% ethanol, rinsed with water and dried overnight. Some plates are coated for 2 hr with 100 ml of cell culture grade type I collagen (50 mg/ml), gelatin (2%) or polylysine (50 mg/ml), all of which can be purchased from Sigma Chemicals (St. Louis, MO) or 10% Matrigel purchased from Becton Dickinson (Bedford, MA), or calf serum, rinsed with PBS and stored at 4 degree C. Cell growth on these plates is assayed by seeding 5,000 cells/well in growth medium and indirect quantitation of cell number through use of alamarBlue as described by the manufacturer Alamar Biosciences, Inc. (Sacramento, CA) after 48 hr. Falcon plate covers #3071 from Becton Dickinson (Bedford, MA) are used to cover the Loprodyne Silent Screen Plates. Falcon Microtest III cell culture plates can also be used in some proliferation experiments.

To prepare extracts, A431 cells are seeded onto the nylon membranes of Loprodyne plates (20,000/200ml/well) and cultured overnight in complete medium. Cells are quiesced by incubation in serum-free basal medium for 24 hr. After 5-20 minutes treatment with EGF (60ng/ml) or a different concentrations of an albumin fusion protein of the invention, the medium was removed and 100 ml of extraction buffer ((20 mM HEPES pH 7.5, 0.15 M NaCl, 1% Triton X-100, 0.1% SDS, 2 mM Na<sub>3</sub>VO<sub>4</sub>, 2 mM Na<sub>4</sub>P<sub>2</sub>O<sub>7</sub> and a cocktail of protease inhibitors (# 1836170) obtained from Boehringer Mannheim (Indianapolis, IN)) is added to each well and the plate is shaken on a rotating shaker for 5 minutes at 4°C. The plate is then placed in a vacuum transfer manifold and the extract filtered through the 0.45 mm membrane bottoms of each well using house

vacuum. Extracts are collected in a 96-well catch/assay plate in the bottom of the vacuum manifold and immediately placed on ice. To obtain extracts clarified by centrifugation, the content of each well, after detergent solubilization for 5 minutes, is removed and centrifuged for 15 minutes at 4 degree C at 16,000 x g.

- 5           Test the filtered extracts for levels of tyrosine kinase activity. Although many methods of detecting tyrosine kinase activity are known, one method is described here.

          Generally, the tyrosine kinase activity of an albumin fusion protein of the invention is evaluated by determining its ability to phosphorylate a tyrosine residue on a specific substrate (a biotinylated peptide). Biotinylated peptides that can be used for this purpose  
10       include PSK1 (corresponding to amino acids 6-20 of the cell division kinase cdc2-p34) and PSK2 (corresponding to amino acids 1-17 of gastrin). Both peptides are substrates for a range of tyrosine kinases and are available from Boehringer Mannheim.

          The tyrosine kinase reaction is set up by adding the following components in order. First, add 10ul of 5uM Biotinylated Peptide, then 10ul ATP/Mg<sub>2</sub><sup>+</sup> (5mM ATP/50mM  
15       MgCl<sub>2</sub>), then 10ul of 5x Assay Buffer (40mM imidazole hydrochloride, pH7.3, 40 mM beta-glycerophosphate, 1mM EGTA, 100mM MgCl<sub>2</sub>, 5 mM MnCl<sub>2</sub>, 0.5 mg/ml BSA), then 5ul of Sodium Vanadate(1mM), and then 5ul of water. Mix the components gently and preincubate the reaction mix at 30 degree C for 2 min. Initiate the reaction by adding 10ul of the control enzyme or the filtered supernatant.

- 20           The tyrosine kinase assay reaction is then terminated by adding 10 ul of 120mM EDTA and place the reactions on ice.

          Tyrosine kinase activity is determined by transferring 50 ul aliquot of reaction mixture to a microtiter plate (MTP) module and incubating at 37 degree C for 20 min. This allows the streptavidin coated 96 well plate to associate with the biotinylated peptide.  
25       Wash the MTP module with 300ul/well of PBS four times. Next add 75 ul of anti-phosphotyrosine antibody conjugated to horse radish peroxidase(anti-P-Tyr-POD(0.5u/ml)) to each well and incubate at 37 degree C for one hour. Wash the well as above.

          Next add 100ul of peroxidase substrate solution (Boehringer Mannheim) and incubate at room temperature for at least 5 mins (up to 30 min). Measure the absorbance  
30       of the sample at 405 nm by using ELISA reader. The level of bound peroxidase activity is quantitated using an ELISA reader and reflects the level of tyrosine kinase activity.

### Example 36: Assay Identifying Phosphorylation Activity

As a potential alternative and/or complement to the assay of protein tyrosine kinase activity described in Example 35, an assay which detects activation (phosphorylation) of major intracellular signal transduction intermediates can also be used. For example, as described below one particular assay can detect tyrosine phosphorylation of the Erk-1 and Erk-2 kinases. However, phosphorylation of other molecules, such as Raf, JNK, p38 MAP, Map kinase kinase (MEK), MEK kinase, Src, Muscle specific kinase (MuSK), IRAK, Tec, and Janus, as well as any other phosphoserine, phosphotyrosine, or phosphothreonine molecule, can be detected by substituting these molecules for Erk-1 or Erk-2 in the following assay.

Specifically, assay plates are made by coating the wells of a 96-well ELISA plate with 0.1ml of protein G (1ug/ml) for 2 hr at room temp, (RT). The plates are then rinsed with PBS and blocked with 3% BSA/PBS for 1 hr at RT. The protein G plates are then treated with 2 commercial monoclonal antibodies (100ng/well) against Erk-1 and Erk-2 (1 hr at RT) (Santa Cruz Biotechnology). (To detect other molecules, this step can easily be modified by substituting a monoclonal antibody detecting any of the above described molecules.) After 3-5 rinses with PBS, the plates are stored at 4 degree C until use.

A431 cells are seeded at 20,000/well in a 96-well Loprodyne filterplate and cultured overnight in growth medium. The cells are then starved for 48 hr in basal medium (DMEM) and then treated with EGF (6ng/well) or varying concentrations of the fusion protein of the invention for 5-20 minutes. The cells are then solubilized and extracts filtered directly into the assay plate.

After incubation with the extract for 1 hr at RT, the wells are again rinsed. As a positive control, a commercial preparation of MAP kinase (10ng/well) is used in place of A431 extract. Plates are then treated with a commercial polyclonal (rabbit) antibody (1ug/ml) which specifically recognizes the phosphorylated epitope of the Erk-1 and Erk-2 kinases (1 hr at RT). This antibody is biotinylated by standard procedures. The bound polyclonal antibody is then quantitated by successive incubations with Europium-streptavidin and Europium fluorescence enhancing reagent in the Wallac DELFIA instrument (time-resolved fluorescence). An increased fluorescent signal over background indicates a phosphorylation by the fusion protein of the present invention or a molecule induced by an albumin fusion protein of the present invention.



**Example 37: Assay for the Stimulation of Bone Marrow CD34+ Cell Proliferation**

This assay is based on the ability of human CD34+ to proliferate in the presence of  
5 hematopoietic growth factors and evaluates the ability of fusion proteins of the invention to stimulate proliferation of CD34+ cells.

It has been previously shown that most mature precursors will respond to only a single signal. More immature precursors require at least two signals to respond. Therefore, to test the effect of fusion proteins of the invention on hematopoietic activity of  
10 a wide range of progenitor cells, the assay contains a given fusion protein of the invention in the presence or absence of hematopoietic growth factors. Isolated cells are cultured for 5 days in the presence of Stem Cell Factor (SCF) in combination with tested sample. SCF alone has a very limited effect on the proliferation of bone marrow (BM) cells, acting in such conditions only as a "survival" factor. However, combined with any factor exhibiting  
15 stimulatory effect on these cells (e.g., IL-3), SCF will cause a synergistic effect. Therefore, if the tested fusion protein has a stimulatory effect on hematopoietic progenitors, such activity can be easily detected. Since normal BM cells have a low level of cycling cells, it is likely that any inhibitory effect of a given fusion protein might not be detected. Accordingly, assays for an inhibitory effect on progenitors is preferably tested in  
20 cells that are first subjected to *in vitro* stimulation with SCF+IL-3, and then contacted with the compound that is being evaluated for inhibition of such induced proliferation.

Briefly, CD34+ cells are isolated using methods known in the art. The cells are thawed and resuspended in medium (QBSF 60 serum-free medium with 1% L-glutamine (500ml) Quality Biological, Inc., Gaithersburg, MD Cat# 160-204-101). After several  
25 gentle centrifugation steps at 200 x g, cells are allowed to rest for one hour. The cell count is adjusted to  $2.5 \times 10^5$  cells/ml. During this time, 100  $\mu$ l of sterile water is added to the peripheral wells of a 96-well plate. The cytokines that can be tested with an albumin fusion protein of the invention in this assay is rhSCF (R&D Systems, Minneapolis, MN, Cat# 255-SC) at 50 ng/ml alone and in combination with rhSCF and rhIL-3 (R&D  
30 Systems, Minneapolis, MN, Cat# 203-ML) at 30 ng/ml. After one hour, 10  $\mu$ l of prepared cytokines, varying concentrations of an albumin fusion protein of the invention, and 20  $\mu$ l of diluted cells are added to the media which is already present in the wells to allow for a

final total volume of 100  $\mu$ l. The plates are then placed in a 37°C/5% CO<sub>2</sub> incubator for five days.

Eighteen hours before the assay is harvested, 0.5  $\mu$ Ci/well of [3H] Thymidine is added in a 10  $\mu$ l volume to each well to determine the proliferation rate. The experiment is terminated by harvesting the cells from each 96-well plate to a filtermat using the Tomtec Harvester 96. After harvesting, the filtermats are dried, trimmed and placed into OmniFilter assemblies consisting of one OmniFilter plate and one OmniFilter Tray. 60  $\mu$ l Microscint is added to each well and the plate sealed with TopSeal-A press-on sealing film. A bar code 15 sticker is affixed to the first plate for counting. The sealed plates are then loaded and the level of radioactivity determined via the Packard Top Count and the printed data collected for analysis. The level of radioactivity reflects the amount of cell proliferation.

The studies described in this example test the activity of a given fusion protein to stimulate bone marrow CD34+ cell proliferation. One skilled in the art could easily modify the exemplified studies to test the activity of fusion proteins and polynucleotides of the invention (e.g., gene therapy) as well as agonists and antagonists thereof. The ability of an albumin fusion protein of the invention to stimulate the proliferation of bone marrow CD34+ cells indicates that the albumin fusion protein and/or polynucleotides corresponding to the fusion protein are useful for the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein.

***Example 38: Assay for Extracellular Matrix Enhanced Cell Response (EMECCR)***

The objective of the Extracellular Matrix Enhanced Cell Response (EMECCR) assay is to evaluate the ability of fusion proteins of the invention to act on hematopoietic stem cells in the context of the extracellular matrix (ECM) induced signal.

Cells respond to the regulatory factors in the context of signal(s) received from the surrounding microenvironment. For example, fibroblasts, and endothelial and epithelial stem cells fail to replicate in the absence of signals from the ECM. Hematopoietic stem cells can undergo self-renewal in the bone marrow, but not in *in vitro* suspension culture. The ability of stem cells to undergo self-renewal *in vitro* is dependent upon their interaction with the stromal cells and the ECM protein fibronectin (fn). Adhesion of cells

to fn is mediated by the  $\alpha_5\beta_1$  and  $\alpha_4\beta_1$  integrin receptors, which are expressed by human and mouse hematopoietic stem cells. The factor(s) which integrate with the ECM environment and are responsible for stimulating stem cell self-renewal have not yet been identified. Discovery of such factors should be of great interest in gene therapy and bone marrow transplant applications

Briefly, polystyrene, non tissue culture treated, 96-well plates are coated with fn fragment at a coating concentration of  $0.2 \mu\text{g}/\text{cm}^2$ . Mouse bone marrow cells are plated (1,000 cells/well) in 0.2 ml of serum-free medium. Cells cultured in the presence of IL-3 (5 ng/ml) + SCF (50 ng/ml) would serve as the positive control, conditions under which little self-renewal but pronounced differentiation of the stem cells is to be expected. Albumin fusion proteins of the invention are tested with appropriate negative controls in the presence and absence of SCF(5.0 ng/ml), where volume of the administered composition containing the albumin fusion protein of the invention represents 10% of the total assay volume. The plated cells are then allowed to grow by incubating in a low oxygen environment (5%  $\text{CO}_2$ , 7%  $\text{O}_2$ , and 88%  $\text{N}_2$ ) tissue culture incubator for 7 days. The number of proliferating cells within the wells is then quantitated by measuring thymidine incorporation into cellular DNA. Verification of the positive hits in the assay will require phenotypic characterization of the cells, which can be accomplished by scaling up of the culture system and using appropriate antibody reagents against cell surface antigens and FACSscan.

One skilled in the art could easily modify the exemplified studies to test the activity of albumin fusion proteins and polynucleotides of the invention (e.g., gene therapy).

If a particular fusion protein of the present invention is found to be a stimulator of hematopoietic progenitors, the fusion protein and polynucleotides corresponding to the fusion protein may be useful for example, in the diagnosis and treatment of disorders affecting the immune system and hematopoiesis. Representative uses are described in the "Immune Activity" and "Infectious Disease" sections above, and elsewhere herein. The fusion protein may also be useful in the expansion of stem cells and committed progenitors of various blood lineages, and in the differentiation and/or proliferation of various cell types.

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Additionally, the albumin fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention, may also be employed to inhibit the proliferation and differentiation of hematopoietic cells and therefore may be employed to protect bone marrow stem cells from chemotherapeutic agents during chemotherapy. This antiproliferative effect may allow administration of higher doses of chemotherapeutic agents and, therefore, more effective chemotherapeutic treatment.

Moreover, fusion proteins of the invention and polynucleotides encoding albumin fusion proteins of the invention may also be useful for the treatment and diagnosis of hematopoietic related disorders such as, anemia, pancytopenia, leukopenia, thrombocytopenia or leukemia, since stromal cells are important in the production of cells of hematopoietic lineages. The uses include bone marrow cell ex-vivo culture, bone marrow transplantation, bone marrow reconstitution, radiotherapy or chemotherapy of neoplasia.

#### **Example 39: Human Dermal Fibroblast and Aortic Smooth Muscle Cell Proliferation**

An albumin fusion protein of the invention is added to cultures of normal human dermal fibroblasts (NHDF) and human aortic smooth muscle cells (AoSMC) and two co-assays are performed with each sample. The first assay examines the effect of the fusion protein on the proliferation of normal human dermal fibroblasts (NHDF) or aortic smooth muscle cells (AoSMC). Aberrant growth of fibroblasts or smooth muscle cells is a part of several pathological processes, including fibrosis, and restenosis. The second assay examines IL6 production by both NHDF and SMC. IL6 production is an indication of functional activation. Activated cells will have increased production of a number of cytokines and other factors, which can result in a proinflammatory or immunomodulatory outcome. Assays are run with and without co-TNF $\alpha$  stimulation, in order to check for costimulatory or inhibitory activity.

Briefly, on day 1, 96-well black plates are set up with 1000 cells/well (NHDF) or 2000 cells/well (AoSMC) in 100  $\mu$ l culture media. NHDF culture media contains: Clonetics FB basal media, 1mg/ml hFGF, 5mg/ml insulin, 50mg/ml gentamycin, 2%FBS, while AoSMC culture media contains Clonetics SM basal media, 0.5  $\mu$ g/ml hEGF, 5mg/ml insulin, 1 $\mu$ g/ml hFGF, 50mg/ml gentamycin, 50  $\mu$ g/ml Amphotericin B, 5%FBS.

After incubation at 37°C for at least 4-5 hours culture media is aspirated and replaced with growth arrest media. Growth arrest media for NHDF contains fibroblast basal media, 50mg/ml gentamycin, 2% FBS, while growth arrest media for AoSMC contains SM basal media, 50mg/ml gentamycin, 50µg/ml Amphotericin B, 0.4% FBS. Incubate at 37 °C until day 2.

On day 2, serial dilutions and templates of an albumin fusion protein of the invention are designed such that they always include media controls and known-protein controls. For both stimulation and inhibition experiments, proteins are diluted in growth arrest media. For inhibition experiments, TNFa is added to a final concentration of 2ng/ml (NHDF) or 5ng/ml (AoSMC). Add 1/3 vol media containing controls or an albumin fusion protein of the invention and incubate at 37 degrees C/5% CO<sub>2</sub> until day 5.

Transfer 60µl from each well to another labeled 96-well plate, cover with a plate-sealer, and store at 4 degrees C until Day 6 (for IL6 ELISA). To the remaining 100 µl in the cell culture plate, aseptically add Alamar Blue in an amount equal to 10% of the culture volume (10µl). Return plates to incubator for 3 to 4 hours. Then measure fluorescence with excitation at 530nm and emission at 590nm using the CytoFluor. This yields the growth stimulation/inhibition data.

On day 5, the IL6 ELISA is performed by coating a 96 well plate with 50-100 ul/well of Anti-Human IL6 Monoclonal antibody diluted in PBS, pH 7.4, incubate ON at room temperature.

On day 6, empty the plates into the sink and blot on paper towels. Prepare Assay Buffer containing PBS with 4% BSA. Block the plates with 200 µl/well of Pierce Super Block blocking buffer in PBS for 1-2 hr and then wash plates with wash buffer (PBS, 0.05% Tween-20). Blot plates on paper towels. Then add 50 µl/well of diluted Anti-Human IL-6 Monoclonal, Biotin-labeled antibody at 0.50 mg/ml. Make dilutions of IL-6 stock in media (30, 10, 3, 1, 0.3, 0 ng/ml). Add duplicate samples to top row of plate. Cover the plates and incubate for 2 hours at RT on shaker.

Plates are washed with wash buffer and blotted on paper towels. Dilute EU-labeled Streptavidin 1:1000 in Assay buffer, and add 100 µl/well. Cover the plate and incubate 1 h at RT. Plates are again washed with wash buffer and blotted on paper towels.

Add 100 µl/well of Enhancement Solution. Shake for 5 minutes. Read the plate on the Wallac DELFIA Fluorometer. Readings from triplicate samples in each assay were

tabulated and averaged.

A positive result in this assay suggests AoSMC cell proliferation and that the albumin fusion protein may be involved in dermal fibroblast proliferation and/or smooth muscle cell proliferation. A positive result also suggests many potential uses of the fusion protein and polynucleotides encoding the albumin fusion protein. For example, inflammation and immune responses, wound healing, and angiogenesis, as detailed throughout this specification. Particularly, fusion proteins may be used in wound healing and dermal regeneration, as well as the promotion of vasculogenesis, both of the blood vessels and lymphatics. The growth of vessels can be used in the treatment of, for example, cardiovascular diseases. Additionally, fusion proteins showing antagonistic activity in this assay may be useful in treating diseases, disorders, and/or conditions which involve angiogenesis by acting as an anti-vascular agent (e.g., anti-angiogenesis). These diseases, disorders, and/or conditions are known in the art and/or are described herein, such as, for example, malignancies, solid tumors, benign tumors, for example hemangiomas, acoustic neuromas, neurofibromas, trachomas, and pyogenic granulomas; arteriosclerotic plaques; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis, retinoblastoma, uveitis and Pterygia (abnormal blood vessel growth) of the eye; rheumatoid arthritis; psoriasis; delayed wound healing; endometriosis; vasculogenesis; granulations; hypertrophic scars (keloids); nonunion fractures; scleroderma; trachoma; vascular adhesions; myocardial angiogenesis; coronary collaterals; cerebral collaterals; arteriovenous malformations; ischemic limb angiogenesis; Osler-Webber Syndrome; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; fibromuscular dysplasia; wound granulation; Crohn's disease; and atherosclerosis. Moreover, albumin fusion proteins that act as antagonists in this assay may be useful in treating anti-hyperproliferative diseases and/or anti-inflammatory known in the art and/or described herein.

#### **Example 40: Cellular Adhesion Molecule (CAM) Expression on Endothelial Cells**

The recruitment of lymphocytes to areas of inflammation and angiogenesis involves specific receptor-ligand interactions between cell surface adhesion molecules

(CAMs) on lymphocytes and the vascular endothelium. The adhesion process, in both normal and pathological settings, follows a multi-step cascade that involves intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and endothelial leukocyte adhesion molecule-1 (E-selectin) expression on endothelial cells (EC). The expression of these molecules and others on the vascular endothelium determines the efficiency with which leukocytes may adhere to the local vasculature and extravasate into the local tissue during the development of an inflammatory response. The local concentration of cytokines and growth factor participate in the modulation of the expression of these CAMs.

Briefly, endothelial cells (e.g., Human Umbilical Vein Endothelial cells (HUVECs)) are grown in a standard 96 well plate to confluence, growth medium is removed from the cells and replaced with 100  $\mu$ l of 199 Medium (10% fetal bovine serum (FBS)). Samples for testing (containing an albumin fusion protein of the invention) and positive or negative controls are added to the plate in triplicate (in 10  $\mu$ l volumes). Plates are then incubated at 37°C for either 5 h (selectin and integrin expression) or 24 h (integrin expression only). Plates are aspirated to remove medium and 100  $\mu$ l of 0.1% paraformaldehyde-PBS(with Ca<sup>++</sup> and Mg<sup>++</sup>) is added to each well. Plates are held at 4°C for 30 min. Fixative is removed from the wells and wells are washed 1X with PBS(+Ca,Mg) + 0.5% BSA and drained. 10  $\mu$ l of diluted primary antibody is added to the test and control wells. Anti-ICAM-1-Biotin, Anti-VCAM-1-Biotin and Anti-E-selectin-Biotin are used at a concentration of 10  $\mu$ g/ml (1:10 dilution of 0.1 mg/ml stock antibody). Cells are incubated at 37°C for 30 min. in a humidified environment. Wells are washed three times with PBS(+Ca,Mg) + 0.5% BSA. 20  $\mu$ l of diluted ExtrAvidin-Alkaline Phosphatase (1:5,000 dilution, referred to herein as the working dilution) are added to each well and incubated at 37°C for 30 min. Wells are washed three times with PBS(+Ca,Mg)+0.5% BSA. Dissolve 1 tablet of p-Nitrophenol Phosphate pNPP per 5 ml of glycine buffer (pH 10.4). 100  $\mu$ l of pNPP substrate in glycine buffer is added to each test well. Standard wells in triplicate are prepared from the working dilution of the ExtrAvidin-Alkaline Phosphatase in glycine buffer: 1:5,000 ( $10^0$ ) >  $10^{-0.5}$  >  $10^{-1}$  >  $10^{-1.5}$ . 5  $\mu$ l of each dilution is added to triplicate wells and the resulting AP content in each well is 5.50 ng, 1.74 ng, 0.55 ng, 0.18 ng. 100  $\mu$ l of pNPP reagent is then added to each of the standard wells. The plate is incubated at 37°C for 4h. A volume of 50  $\mu$ l of 3M NaOH is

added to all wells. The plate is read on a plate reader at 405 nm using the background subtraction option on blank wells filled with glycine buffer only. Additionally, the template is set up to indicate the concentration of AP-conjugate in each standard well [ 5.50 ng; 1.74 ng; 0.55 ng; 0.18 ng]. Results are indicated as amount of bound AP-conjugate in each sample.

***Example 41: Alamar Blue Endothelial Cells Proliferation Assay***

This assay may be used to quantitatively determine protein mediated inhibition of bFGF-induced proliferation of Bovine Lymphatic Endothelial Cells (LECs), Bovine Aortic Endothelial Cells (BAECs) or Human Microvascular Uterine Myometrial Cells (UTMECs). This assay incorporates a fluorometric growth indicator based on detection of metabolic activity. A standard Alamar Blue Proliferation Assay is prepared in EGM-2MV with 10 ng /ml of bFGF added as a source of endothelial cell stimulation. This assay may be used with a variety of endothelial cells with slight changes in growth medium and cell concentration. Dilutions of protein batches to be tested are diluted as appropriate. Serum-free medium (GIBCO SFM) without bFGF is used as a non-stimulated control and Angiostatin or TSP-1 are included as a known inhibitory controls.

Briefly, LEC, BAECs or UTMECs are seeded in growth media at a density of 5000 to 2000 cells/well in a 96 well plate and placed at 37 degreesC overnight. After the overnight incubation of the cells, the growth media is removed and replaced with GIBCO EC-SFM. The cells are treated with the appropriate dilutions of an albumin fusion protein of the invention or control protein sample(s) (prepared in SFM ) in triplicate wells with additional bFGF to a concentration of 10 ng/ ml. Once the cells have been treated with the samples, the plate(s) is/are placed back in the 37° C incubator for three days. After three days 10 ml of stock alamar blue (Biosource Cat# DAL1100) is added to each well and the plate(s) is/are placed back in the 37°C incubator for four hours. The plate(s) are then read at 530nm excitation and 590nm emission using the CytoFluor fluorescence reader. Direct output is recorded in relative fluorescence units.

Alamar blue is an oxidation-reduction indicator that both fluoresces and changes color in response to chemical reduction of growth medium resulting from cell growth. As cells grow in culture, innate metabolic activity results in a chemical reduction of the immediate surrounding environment. Reduction related to growth causes the indicator to



change from oxidized (non-fluorescent blue) form to reduced (fluorescent red) form (i.e., stimulated proliferation will produce a stronger signal and inhibited proliferation will produce a weaker signal and the total signal is proportional to the total number of cells as well as their metabolic activity). The background level of activity is observed with the starvation medium alone. This is compared to the output observed from the positive control samples (bFGF in growth medium) and protein dilutions.

***Example 42: Detection of Inhibition of a Mixed Lymphocyte Reaction***

This assay can be used to detect and evaluate inhibition of a Mixed Lymphocyte Reaction (MLR) by fusion proteins of the invention. Inhibition of a MLR may be due to a direct effect on cell proliferation and viability, modulation of costimulatory molecules on interacting cells, modulation of adhesiveness between lymphocytes and accessory cells, or modulation of cytokine production by accessory cells. Multiple cells may be targeted by the albumin fusion proteins that inhibit MLR since the peripheral blood mononuclear fraction used in this assay includes T, B and natural killer lymphocytes, as well as monocytes and dendritic cells.

Albumin fusion proteins of the invention found to inhibit the MLR may find application in diseases associated with lymphocyte and monocyte activation or proliferation. These include, but are not limited to, diseases such as asthma, arthritis, diabetes, inflammatory skin conditions, psoriasis, eczema, systemic lupus erythematosus, multiple sclerosis, glomerulonephritis, inflammatory bowel disease, crohn's disease, ulcerative colitis, arteriosclerosis, cirrhosis, graft vs. host disease, host vs. graft disease, hepatitis, leukemia and lymphoma.

Briefly, PBMCs from human donors are purified by density gradient centrifugation using Lymphocyte Separation Medium (LSM<sup>®</sup>, density 1.0770 g/ml, Organon Teknika Corporation, West Chester, PA). PBMCs from two donors are adjusted to  $2 \times 10^6$  cells/ml in RPMI-1640 (Life Technologies, Grand Island, NY) supplemented with 10% FCS and 2 mM glutamine. PBMCs from a third donor is adjusted to  $2 \times 10^5$  cells/ml. Fifty microliters of PBMCs from each donor is added to wells of a 96-well round bottom microtiter plate. Dilutions of the fusion protein test material (50  $\mu$ l) is added in triplicate to microtiter wells. Test samples (of the protein of interest) are added for final dilution of 1:4; rhuIL-2 (R&D Systems, Minneapolis, MN, catalog number 202-IL) is added to a final

concentration of 1 µg/ml; anti-CD4 mAb (R&D Systems, clone 34930.11, catalog number MAB379) is added to a final concentration of 10 µg/ml. Cells are cultured for 7-8 days at 37°C in 5% CO<sub>2</sub>, and 1 µC of [<sup>3</sup>H] thymidine is added to wells for the last 16 hrs of culture. Cells are harvested and thymidine incorporation determined using a Packard  
5 TopCount. Data is expressed as the mean and standard deviation of triplicate determinations.

Samples of the fusion protein of interest are screened in separate experiments and compared to the negative control treatment, anti-CD4 mAb, which inhibits proliferation of lymphocytes and the positive control treatment, IL-2 (either as recombinant material or  
10 supernatant), which enhances proliferation of lymphocytes.

#### ***Example 43: Assays for Protease Activity***

The following assay may be used to assess protease activity of an albumin fusion protein of the invention.

15 Gelatin and casein zymography are performed essentially as described (Heusen et al., *Anal. Biochem.*, 102:196-202 (1980); Wilson et al., *Journal of Urology*, 149:653-658 (1993)). Samples are run on 10% polyacrylamide/0.1% SDS gels containing 1% gelatin or casein, soaked in 2.5% triton at room temperature for 1 hour, and in 0.1M glycine, pH 8.3 at 37°C 5 to 16 hours. After staining in amido black areas of proteolysis appear as clear  
20 areas against the blue-black background. Trypsin (Sigma T8642) is used as a positive control.

Protease activity is also determined by monitoring the cleavage of n-a-benzoyl-L-arginine ethyl ester (BAEE) (Sigma B-4500). Reactions are set up in (25mMNaPO<sub>4</sub>, 1mM EDTA, and 1mM BAEE), pH 7.5. Samples are added and the change in adsorbance at  
25 260nm is monitored on the Beckman DU-6 spectrophotometer in the time-drive mode. Trypsin is used as a positive control.

Additional assays based upon the release of acid-soluble peptides from casein or hemoglobin measured as adsorbance at 280 nm or colorimetrically using the Folin method are performed as described in Bergmeyer, et al., *Methods of Enzymatic Analysis*, 5 (1984).

30 Other assays involve the solubilization of chromogenic substrates (Ward, *Applied Science*, 251-317 (1983)).

**Example 44: Identifying Serine Protease Substrate Specificity**

Methods known in the art or described herein may be used to determine the substrate specificity of the albumin fusion proteins of the present invention having serine protease activity. A preferred method of determining substrate specificity is by the use of positional scanning synthetic combinatorial libraries as described in GB 2 324 529 (incorporated herein in its entirety).

**Example 45: Ligand Binding Assays**

The following assay may be used to assess ligand binding activity of an albumin fusion protein of the invention.

Ligand binding assays provide a direct method for ascertaining receptor pharmacology and are adaptable to a high throughput format. The purified ligand for an albumin fusion protein of the invention is radiolabeled to high specific activity (50-2000 Ci/mmol) for binding studies. A determination is then made that the process of radiolabeling does not diminish the activity of the ligand towards the fusion protein. Assay conditions for buffers, ions, pH and other modulators such as nucleotides are optimized to establish a workable signal to noise ratio for both membrane and whole cell polypeptide sources. For these assays, specific polypeptide binding is defined as total associated radioactivity minus the radioactivity measured in the presence of an excess of unlabeled competing ligand. Where possible, more than one competing ligand is used to define residual nonspecific binding.

**Example 46: Functional Assay in *Xenopus* Oocytes**

Capped RNA transcripts from linearized plasmid templates encoding an albumin fusion protein of the invention is synthesized in vitro with RNA polymerases in accordance with standard procedures. In vitro transcripts are suspended in water at a final concentration of 0.2 mg/ml. Ovarian lobes are removed from adult female toads, Stage V defolliculated oocytes are obtained, and RNA transcripts (10 ng/oocyte) are injected in a 50 nl bolus using a microinjection apparatus. Two electrode voltage clamps are used to measure the currents from individual *Xenopus oocytes* in response fusion protein and polypeptide agonist exposure. Recordings are made in Ca<sup>2+</sup> free Barth's medium at room

temperature. The *Xenopus* system can be used to screen known ligands and tissue/cell extracts for activating ligands.

***Example 47: Microphysiometric Assays***

5       Activation of a wide variety of secondary messenger systems results in extrusion of small amounts of acid from a cell. The acid formed is largely as a result of the increased metabolic activity required to fuel the intracellular signaling process. The pH changes in the media surrounding the cell are very small but are detectable by the CYTOSENSOR microphysiometer (Molecular Devices Ltd., Menlo Park, Calif.). The CYTOSENSOR is  
10       thus capable of detecting the ability of an albumin fusion protein of the invention to activate secondary messengers that are coupled to an energy utilizing intracellular signaling pathway.

***Example 48: Extract/Cell Supernatant Screening***

15       A large number of mammalian receptors exist for which there remains, as yet, no cognate activating ligand (agonist). Thus, active ligands for these receptors may not be included within the ligands banks as identified to date. Accordingly, the albumin fusion proteins of the invention can also be functionally screened (using calcium, cAMP, microphysiometer, oocyte electrophysiology, etc., functional screens) against tissue  
20       extracts to identify natural ligands for the Therapeutic protein portion and/or albumin protein portion of an albumin fusion protein of the invention. Extracts that produce positive functional responses can be sequentially subfractionated until an activating ligand is isolated and identified.

***Example 49: ATP-binding assay***

25       The following assay may be used to assess ATP-binding activity of fusion proteins of the invention.

ATP-binding activity of an albumin fusion protein of the invention may be detected using the ATP-binding assay described in U.S. Patent 5,858,719, which is herein  
30       incorporated by reference in its entirety. Briefly, ATP-binding to an albumin fusion protein of the invention is measured via photoaffinity labeling with 8-azido-ATP in a competition assay. Reaction mixtures containing 1 mg/ml of ABC transport protein are incubated with

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varying concentrations of ATP, or the non-hydrolyzable ATP analog adenylyl-5'-imidodiphosphate for 10 minutes at 4°C. A mixture of 8-azido-ATP (Sigma Chem. Corp., St. Louis, MO.) plus 8-azido-ATP (<sup>32</sup>P-ATP) (5 mCi/μmol, ICN, Irvine CA.) is added to a final concentration of 100 μM and 0.5 ml aliquots are placed in the wells of a porcelain spot plate on ice. The plate is irradiated using a short wave 254 nm UV lamp at a distance of 2.5 cm from the plate for two one-minute intervals with a one-minute cooling interval in between. The reaction is stopped by addition of dithiothreitol to a final concentration of 2mM. The incubations are subjected to SDS-PAGE electrophoresis, dried, and autoradiographed. Protein bands corresponding to the albumin fusion proteins of the invention are excised, and the radioactivity quantified. A decrease in radioactivity with increasing ATP or adenylyl-5'-imidodiphosphate provides a measure of ATP affinity to the fusion protein.

***Example 50: Phosphorylation Assay***

In order to assay for phosphorylation activity of an albumin fusion protein of the invention, a phosphorylation assay as described in U.S. Patent 5,958,405 (which is herein incorporated by reference) is utilized. Briefly, phosphorylation activity may be measured by phosphorylation of a protein substrate using gamma-labeled <sup>32</sup>P-ATP and quantitation of the incorporated radioactivity using a gamma radioisotope counter. The fusion protein of the invention is incubated with the protein substrate, <sup>32</sup>P-ATP, and a kinase buffer. The <sup>32</sup>P incorporated into the substrate is then separated from free <sup>32</sup>P-ATP by electrophoresis, and the incorporated <sup>32</sup>P is counted and compared to a negative control. Radioactivity counts above the negative control are indicative of phosphorylation activity of the fusion protein.

***Example 51: Detection of Phosphorylation Activity (Activation) of an Albumin Fusion Protein of the Invention in the Presence of Polypeptide Ligands***

Methods known in the art or described herein may be used to determine the phosphorylation activity of an albumin fusion protein of the invention. A preferred method of determining phosphorylation activity is by the use of the tyrosine phosphorylation assay as described in US 5,817,471 (incorporated herein by reference).

**Example 52: Identification Of Signal Transduction Proteins That Interact With An albumin fusion protein Of The Present Invention**

Albumin fusion proteins of the invention may serve as research tools for the identification, characterization and purification of signal transduction pathway proteins or  
5 receptor proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as carcinoma tissues, is passed over the column, and molecules with appropriate affinity bind  
10 to the albumin fusion protein. The protein complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

**Example 53: IL-6 Bioassay**

A variety of assays are known in the art for testing the proliferative effects of an albumin fusion protein of the invention. For example, one such assay is the IL-6 Bioassay as described by Marz *et al.* (*Proc. Natl. Acad. Sci., U.S.A.*, 95:3251-56 (1998)), which is herein incorporated by reference). After 68 hrs. at 37°C, the number of viable  
20 cells is measured by adding the tetrazolium salt thiazolyl blue (MTT) and incubating for a further 4 hrs. at 37°C. B9 cells are lysed by SDS and optical density is measured at 570 nm. Controls containing IL-6 (positive) and no cytokine (negative) are Briefly, IL-6 dependent B9 murine cells are washed three times in IL-6 free medium and plated at a concentration of 5,000 cells per well in 50 µl, and 50 µl of fusion protein of the invention  
25 is added. utilized. Enhanced proliferation in the test sample(s) (containing an albumin fusion protein of the invention) relative to the negative control is indicative of proliferative effects mediated by the fusion protein.

**Example 54: Support of Chicken Embryo Neuron Survival**

30 To test whether sympathetic neuronal cell viability is supported by an albumin fusion protein of the invention, the chicken embryo neuronal survival assay of Senaldi *et al* may be utilized (*Proc. Natl. Acad. Sci., U.S.A.*, 96:11458-63 (1998)), which is herein

incorporated by reference). Briefly, motor and sympathetic neurons are isolated from chicken embryos, resuspended in L15 medium (with 10% FCS, glucose, sodium selenite, progesterone, conalbumin, putrescine, and insulin; Life Technologies, Rockville, MD.) and Dulbecco's modified Eagles medium [with 10% FCS, glutamine, penicillin, and 25 mM Hepes buffer (pH 7.2); Life Technologies, Rockville, MD.], respectively, and incubated at 37°C in 5% CO<sub>2</sub> in the presence of different concentrations of the purified fusion protein of the invention, as well as a negative control lacking any cytokine. After 3 days, neuron survival is determined by evaluation of cellular morphology, and through the use of the colorimetric assay of Mosmann (Mosmann, T., *J. Immunol. Methods*, 65:55-63. (1983)). Enhanced neuronal cell viability as compared to the controls lacking cytokine is indicative of the ability of the albumin fusion protein to enhance the survival of neuronal cells.

***Example 55: Assay for Phosphatase Activity***

The following assay may be used to assess serine/threonine phosphatase (PTPase) activity of an albumin fusion protein of the invention.

In order to assay for serine/threonine phosphatase (PTPase) activity, assays can be utilized which are widely known to those skilled in the art. For example, the serine/threonine phosphatase (PSPase) activity of an albumin fusion protein of the invention may be measured using a PSPase assay kit from New England Biolabs, Inc. Myelin basic protein (MyBP), a substrate for PSPase, is phosphorylated on serine and threonine residues with cAMP-dependent Protein Kinase in the presence of [<sup>32</sup>P]ATP. Protein serine/threonine phosphatase activity is then determined by measuring the release of inorganic phosphate from 32P-labeled MyBP.

***Example 56: Interaction of Serine/Threonine Phosphatases with other Proteins***

Fusion protein of the invention having serine/threonine phosphatase activity ( e.g., as determined in Example 55) are useful, for example, as research tools for the identification, characterization and purification of additional interacting proteins or receptor proteins, or other signal transduction pathway proteins. Briefly, a labeled fusion protein of the invention is useful as a reagent for the purification of molecules with which it interacts. In one embodiment of affinity purification, an albumin fusion protein of the

invention is covalently coupled to a chromatography column. Cell-free extract derived from putative target cells, such as neural or liver cells, is passed over the column, and molecules with appropriate affinity bind to the fusion protein. The fusion protein -complex is recovered from the column, dissociated, and the recovered molecule subjected to N-terminal protein sequencing. This amino acid sequence is then used to identify the captured molecule or to design degenerate oligonucleotide probes for cloning the relevant gene from an appropriate cDNA library.

***Example 57: Assaying for Heparanase Activity***

There are numerous assays known in the art that may be employed to assay for heparanase activity of an albumin fusion protein of the invention. In one example, heparanase activity of an albumin fusion protein of the invention, is assayed as described by Vlodavsky et al., (Vlodavsky et al., Nat. Med., 5:793-802 (1999)). Briefly, cell lysates, conditioned media, intact cells ( $1 \times 10^6$  cells per 35-mm dish), cell culture supernatant, or purified fusion protein are incubated for 18 hrs at 37°C, pH 6.2-6.6, with  $^{35}\text{S}$ -labeled ECM or soluble ECM derived peak I proteoglycans. The incubation medium is centrifuged and the supernatant is analyzed by gel filtration on a Sepharose CL-6B column (0.9 x 30 cm). Fractions are eluted with PBS and their radioactivity is measured. Degradation fragments of heparan sulfate side chains are eluted from Sepharose 6B at  $0.5 < K_{av} < 0.8$  (peak II). Each experiment is done at least three times. Degradation fragments corresponding to "peak II," as described by Vlodavsky et al., is indicative of the activity of an albumin fusion protein of the invention in cleaving heparan sulfate.

***Example 58: Immobilization of biomolecules***

This example provides a method for the stabilization of an albumin fusion protein of the invention in non-host cell lipid bilayer constructs (see, e.g., Bieri et al., Nature Biotech 17:1105-1108 (1999), hereby incorporated by reference in its entirety herein) which can be adapted for the study of fusion proteins of the invention in the various functional assays described above. Briefly, carbohydrate-specific chemistry for biotinylation is used to confine a biotin tag to an albumin fusion protein of the invention, thus allowing uniform orientation upon immobilization. A 50uM solution of an albumin fusion protein of the invention in washed membranes is incubated with 20 mM NaIO<sub>4</sub> and



1.5 mg/ml (4mM) BACH or 2 mg/ml (7.5mM) biotin-hydrazide for 1 hr at room temperature (reaction volume, 150ul). Then the sample is dialyzed (Pierce Slidealizer Cassett, 10 kDa cutoff; Pierce Chemical Co., Rockford IL) at 4C first for 5 h, exchanging the buffer after each hour, and finally for 12 h against 500 ml buffer R (0.15 M NaCl, 1 mM MgCl<sub>2</sub>, 10 mM sodium phosphate, pH7). Just before addition into a cuvette, the sample is diluted 1:5 in buffer ROG50 (Buffer R supplemented with 50 mM octylglucoside).

#### *Example 59: Assays for Metalloproteinase Activity*

Metalloproteinases are peptide hydrolases which use metal ions, such as Zn<sup>2+</sup>, as the catalytic mechanism. Metalloproteinase activity of an albumin fusion protein of the present invention can be assayed according to methods known in the art. The following exemplary methods are provided:

##### *Proteolysis of alpha-2-macroglobulin*

To confirm protease activity, a purified fusion protein of the invention is mixed with the substrate alpha-2-macroglobulin (0.2 unit/ml; Boehringer Mannheim, Germany) in 1x assay buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl<sub>2</sub>, 25 μM ZnCl<sub>2</sub> and 0.05% Brij-35) and incubated at 37°C for 1-5 days. Trypsin is used as positive control. Negative controls contain only alpha-2-macroglobulin in assay buffer. The samples are collected and boiled in SDS-PAGE sample buffer containing 5% 2-mercaptoethanol for 5-min, then loaded onto 8% SDS-polyacrylamide gel. After electrophoresis the proteins are visualized by silver staining. Proteolysis is evident by the appearance of lower molecular weight bands as compared to the negative control.

##### *Inhibition of alpha-2-macroglobulin proteolysis by inhibitors of metalloproteinases*

Known metalloproteinase inhibitors (metal chelators (EDTA, EGTA, AND HgCl<sub>2</sub>), peptide metalloproteinase inhibitors (TIMP-1 and TIMP-2), and commercial small molecule MMP inhibitors) may also be used to characterize the proteolytic activity of an albumin fusion protein of the invention. Three synthetic MMP inhibitors that may be used are: MMP inhibitor I, [IC<sub>50</sub> = 1.0 μM against MMP-1 and MMP-8; IC<sub>50</sub> = 30 μM against MMP-9; IC<sub>50</sub> = 150 μM against MMP-3]; MMP-3 (stromelysin-1) inhibitor I [IC<sub>50</sub>

= 5  $\mu$ M against MMP-3], and MMP-3 inhibitor II [ $K_i$  = 130 nM against MMP-3]; inhibitors available through Calbiochem, catalog # 444250, 444218, and 444225, respectively). Briefly, different concentrations of the small molecule MMP inhibitors are mixed with a purified fusion protein of the invention (50 $\mu$ g/ml) in 22.9  $\mu$ l of 1x HEPES buffer (50 mM HEPES, pH 7.5, 0.2 M NaCl, 10 mM CaCl<sub>2</sub>, 25  $\mu$ M ZnCl<sub>2</sub> and 0.05%Brij-35) and incubated at room temperature (24 °C) for 2-hr, then 7.1  $\mu$ l of substrate alpha-2-macroglobulin (0.2 unit/ml) is added and incubated at 37°C for 20-hr. The reactions are stopped by adding 4x sample buffer and boiled immediately for 5 minutes. After SDS-PAGE, the protein bands are visualized by silver stain.

#### *Synthetic Fluorogenic Peptide Substrates Cleavage Assay*

The substrate specificity for fusion proteins of the invention with demonstrated metalloproteinase activity may be determined using techniques known in the art, such as using synthetic fluorogenic peptide substrates (purchased from BACHEM Bioscience Inc). Test substrates include, M-1985, M-2225, M-2105, M-2110, and M-2255. The first four are MMP substrates and the last one is a substrate of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) converting enzyme (TACE). These substrates are preferably prepared in 1:1 dimethyl sulfoxide (DMSO) and water. The stock solutions are 50-500  $\mu$ M. Fluorescent assays are performed by using a Perkin Elmer LS 50B luminescence spectrometer equipped with a constant temperature water bath. The excitation  $\lambda$  is 328 nm and the emission  $\lambda$  is 393 nm. Briefly, the assay is carried out by incubating 176  $\mu$ l 1x HEPES buffer (0.2 M NaCl, 10 mM CaCl<sub>2</sub>, 0.05% Brij-35 and 50 mM HEPES, pH 7.5) with 4  $\mu$ l of substrate solution (50  $\mu$ M) at 25 °C for 15 minutes, and then adding 20  $\mu$ l of a purified fusion protein of the invention into the assay cuvet. The final concentration of substrate is 1  $\mu$ M. Initial hydrolysis rates are monitored for 30-min.

#### *Example 60: Identification and Cloning of VH and VL domains*

One method to identify and clone VH and VL domains from cell lines expressing a particular antibody is to perform PCR with VH and VL specific primers on cDNA made from the antibody expressing cell lines. Briefly, RNA is isolated from the cell lines and used as a template for RT-PCR designed to amplify the VH and VL domains of the antibodies expressed by the EBV cell lines. Cells may be lysed in the TRIzol® reagent

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(Life Technologies, Rockville, MD) and extracted with one fifth volume of chloroform. After addition of chloroform, the solution is allowed to incubate at room temperature for 10 minutes, and the centrifuged at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. The supernatant is collected and RNA is precipitated using an equal volume of isopropanol. Precipitated RNA is pelleted by centrifuging at 14,000 rpm for 15 minutes at 4°C in a tabletop centrifuge. Following centrifugation, the supernatant is discarded and washed with 75% ethanol. Following washing, the RNA is centrifuged again at 800 rpm for 5 minutes at 4°C. The supernatant is discarded and the pellet allowed to air dry. RNA is dissolved in DEPC water and heated to 60°C for 10 minutes. Quantities of RNA can be determined using optical density measurements.

cDNA may be synthesized, according to methods well-known in the art, from 1.5-2.5 micrograms of RNA using reverse transcriptase and random hexamer primers. cDNA is then used as a template for PCR amplification of VH and VL domains. Primers used to amplify VH and VL genes are shown in Table 3. Typically a PCR reaction makes use of a single 5' primer and a single 3' primer. Sometimes, when the amount of available RNA template is limiting, or for greater efficiency, groups of 5' and/or 3' primers may be used. For example, sometimes all five VH-5' primers and all JH3' primers are used in a single PCR reaction. The PCR reaction is carried out in a 50 microliter volume containing 1X PCR buffer, 2mM of each dNTP, 0.7 units of High Fidelity Taq polymerase, 5' primer mix, 3' primer mix and 7.5 microliters of cDNA. The 5' and 3' primer mix of both VH and VL can be made by pooling together 22 pmole and 28 pmole, respectively, of each of the individual primers. PCR conditions are: 96°C for 5 minutes; followed by 25 cycles of 94°C for 1 minute, 50°C for 1 minute, and 72°C for 1 minute; followed by an extension cycle of 72°C for 10 minutes. After the reaction is completed, sample tubes are stored at 4°C.

Table 3: Primer Sequences Used to Amplify VH and VL domains.

	Primer name	SEQ ID NO	Primer Sequence (5'-3')
	VH Primers		
5	Hu VH1-5'	36	CAGGTGCAGCTGGTGCAGTCTGG
	Hu VH2-5'	37	CAGGTCAACTTAAGGGAGTCTGG
	Hu VH3-5'	38	GAGGTGCAGCTGGTGGAGTCTGG
	Hu VH4-5'	39	CAGGTGCAGCTGCAGGAGTCCGGG
	Hu VH5-5'	40	GAGGTGCAGCTGTTGCAGTCTGC
10	Hu VH6-5'	41	CAGGTACAGCTGCAGCAGTCAGG
	Hu JH1,2-5'	42	TGAGGAGACGGTGACCAGGGTGCC
	Hu JH3-5'	43	TGAAGAGACGGTGACCATTGTCCC
	Hu JH4,5-5'	44	TGAGGAGACGGTGACCAGGGTTCC
	Hu JH6-5'	45	TGAGGAGACGGTGACCGTGGTCCC
15	VL Primers		
	Hu Vkappa1-5'	46	GACATCCAGATGACCCAGTCTCC
	Hu Vkappa2a-5'	47	GATGTTGTGATGACTCAGTCTCC
	Hu Vkappa2b-5'	48	GATATTGTGATGACTCAGTCTCC
20	Hu Vkappa3-5'	49	GAAATTGTGTTGACGCAGTCTCC
	Hu Vkappa4-5'	50	GACATCGTGATGACCCAGTCTCC
	Hu Vkappa5-5'	51	GAAACGACACTCACGCAGTCTCC
	Hu Vkappa6-5'	52	GAAATTGTGCTGACTCAGTCTCC
	Hu Vlambda1-5'	53	CAGTCTGTGTTGACGCAGCCGCC
25	Hu Vlambda2-5'	54	CAGTCTGCCCTGACTCAGCCTGC
	Hu Vlambda3-5'	55	TCCTATGTGCTGACTCAGCCACC
	Hu Vlambda3b-5'	56	TCTTCTGAGCTGACTCAGGACCC
	Hu Vlambda4-5'	57	CACGTTATACTGACTCAACCGCC
	Hu Vlambda5-5'	58	CAGGCTGTGCTCACTCAGCCGTC
30	Hu Vlambda6-5'	59	AATTTTATGCTGACTCAGCCCCA
	Hu Jkappa1-3'	60	ACGTTTGATTTCCACCTTGGTCCC
	Hu Jkappa2-3'	61	ACGTTTGATCTCCAGCTTGGTCCC
	Hu Jkappa3-3'	62	ACGTTTGATATCCACTTTGGTCCC
	Hu Jkappa4-3'	63	ACGTTTGATCTCCACCTTGGTCCC
35	Hu Jkappa5-3'	64	ACGTTTAATCTCCAGTCGTGTCCC
	Hu Jlambda1-3'	65	CAGTCTGTGTTGACGCAGCCGCC
	Hu Jlambda2-3'	66	CAGTCTGCCCTGACTCAGCCTGC
	Hu Jlambda3--3'	67	TCCTATGTGCTGACTCAGCCACC
	Hu Jlambda3b-3'	68	TCTTCTGAGCTGACTCAGGACCC
40	Hu Jlambda4-3'	69	CACGTTATACTGACTCAACCGCC
	Hu Jlambda5-3'	70	CAGGCTGTGCTCACTCAGCCGTC
	Hu Jlambda6-3'	71	AATTTTATGCTGACTCAGCCCCA

PCR samples are then electrophoresed on a 1.3% agarose gel. DNA bands of the expected sizes (~506 base pairs for VH domains, and 344 base pairs for VL domains) can be cut out of the gel and purified using methods well known in the art. Purified PCR products can be ligated into a PCR cloning vector (TA vector from Invitrogen Inc.,  
5 Carlsbad, CA). Individual cloned PCR products can be isolated after transfection of E. coli and blue/white color selection. Cloned PCR products may then be sequenced using methods commonly known in the art.

The PCR bands containing the VH domain and the VL domains can also be used to create full-length Ig expression vectors. VH and VL domains can be cloned into vectors  
10 containing the nucleotide sequences of a heavy (e.g., human IgG1 or human IgG4) or light chain (human kappa or human lambda) constant regions such that a complete heavy or light chain molecule could be expressed from these vectors when transfected into an appropriate host cell. Further, when cloned heavy and light chains are both expressed in one cell line (from either one or two vectors), they can assemble into a complete functional  
15 antibody molecule that is secreted into the cell culture medium. Methods using polynucleotides encoding VH and VL antibody domain to generate expression vectors that encode complete antibody molecules are well known within the art.

It will be clear that the invention may be practiced otherwise than as particularly  
20 described in the foregoing description and examples. Numerous modifications and variations of the present invention are possible in light of the above teachings and, therefore, are within the scope of the appended claims.

The entire disclosure of each document cited (including patents, patent  
25 applications, patent publications, journal articles, abstracts, laboratory manuals, books, or other disclosures) as well as information available through Identifiers specific to databases such as GenBank, GeneSeq, or the CAS Registry, referred to in this application are herein incorporated by reference in their entirety. The specification and sequence listing of each of the following U.S. applications are herein incorporated by reference in their entirety:

30

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